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Simeon Noel Tibbo (1914–2003)

A well-known and highly respected fisheries scientist in Atlantic Canada, S. Noel Tibbo died on 11 January 2003 at his home in Nanaimo, British Columbia.

Noel was born in Grand Bank, Newfoundland, and gained his early education at school there and at Memorial University of Newfoundland (St. John's, Newfoundland and Labrador), before moving to Acadia University (Wolfville, Nova Scotia) to take his B. Sc. (Hons.) in 1940. His career in fisheries opened with a position as Assistant Scientist with the Department of Natural Resources in St. John's, Newfoundland and Labrador, where he was involved in research on several marine species, including the Atlantic herring (*Clupea harengus*), which was to play a major part in his future career. At the same time, he held a graduate fellowship at the University of Toronto (Ontario) from 1943 to 1946 to earn his M.A. degree.

Noel's long association with St. Andrews, New Brunswick, began in 1946 with his appointment as a scientist to the Atlantic Herring Investigation Committee, with special interest in the biology of and fisheries for Atlantic herring in the Gulf of St. Lawrence. He worked with the Committee until 1950, when he transferred to the Fisheries Research Board of Canada (later to become Fisheries and Oceans Canada) as Scientist-in-Charge of the Pelagic Section of the Marine Fish Division. Atlantic herring was the principal interest of his group, with responsibilities including biology, stock structure, population dynamics, and recommendations for management of the species from the Gulf of St. Lawrence to the Gulf of Maine and the Bay of Fundy.

During the first 20 years of his career, Noel published at least 35 scientific papers on the biology and management of Atlantic herring. In fact, his interest in and scientific contributions to our knowledge of the Atlantic herring continued throughout his professional life. During his early Newfoundland investigations and as part of his M.A. thesis, Tibbo made the significant conclusion that based on vertebral counts and other information, herring populations on the south and west coasts of Newfoundland were separate and distinct (S.N. Tibbo. 1956. J. Fish. Res. Board Can. **13**: 449–466). Noel's conclusions were subsequently verified through mark and recapture studies (J. Wheeler, Fisheries and Oceans Canada, Northwest Fisheries Science Centre, St. John's, Newfoundland and Labrador, personal communication). Noel's studies of different populations of Atlantic herring (S.N. Messieh and S.N. Tibbo. 1971. J. Fish. Res. Board Can. **28**: 1009–1014) in the Gulf of St. Lawrence were also

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noteworthy, in that he laid the foundation for the current stock assessments that treat spring and fall spawning components separately (G. Chouinard, Fisheries and Oceans Canada, Gulf Fisheries Centre, Moncton, New Brunswick, personal communication). In these ways, his contributions are early examples of the work of Canadian scientists that identified the biodiversity of marine fish stocks.

The Pelagic Section under Noel Tibbo also conducted numerous studies on the biology and fisheries for such pelagic fish as swordfish (*Xiphias gladius*), Atlantic mackerel (*Scomber scombrus*), and tunas, particularly bluefin tuna (*Thunnus thynnus*), and some species of sharks. The swordfish investigations were significant because the fishery was rapidly expanding from an opportunistic harpoon fishery to a regular, species-directed, longline fishery. Studies included monitoring commercial catches and general biology of swordfish, such as distribution in Canadian waters, seasonal migration, food, and reproduction. Noel's involvement was significant and led to seven additional publications, including a frequently cited Fisheries Research Board Bulletin (S.N. Tibbo, L.R. Day, and W.F. Doucet. 1961. Fish. Res. Board Bull. No. 130) that provided a synthesis of the biology of swordfish and its Canadian fishery.

When Noel's research contributions are considered in sum, the breadth of his work is striking. Beyond his studies of herring and swordfish, Noel also contributed to our understanding of the diverse pelagic fish fauna of Atlantic Canadian waters, including pelagic stingray (*Pteroplatytrigon violacea*, formerly *Dasyatis violacea*; W.B. Scott and S.N. Tibbo. 1968. J. Fish. Res. Board Can. **25**: 1075–1076), opah (*Lampris guttatus*; R.A. McKenzie and S.N. Tibbo. 1963. J. Fish. Res. Board Can. **20**: 1097–1099), dolphinfish (*Coryphaena hippurus*; S.N. Tibbo. 1962. J. Fish. Res. Board Can. **19**: 517–518), as well as dusky shark (*Carcharhinus obscurus*; S.N. Tibbo and R.A. McKenzie. 1963. J. Fish. Res. Board Can. **20**: 1101–1102), shortfin mako (*Isurus oxyrinchus*; S.N. Tibbo, R.A. McKenzie, and W.B. Scott. 1963. J. Fish. Res. Board Can. **20**: 1343–1354), oceanic whitetip shark (*Carcharhinus longimanus*; S.N. Tibbo. 1962. J. Fish. Res. Board Can. **19**: 517–518), and porbeagle (*Lamna nasus*; R.A. McKenzie and S.N. Tibbo. 1964. J. Fish. Res. Board Can. **21**: 863–864).

Responsibility for pelagic fisheries meant Noel was frequently required to work at sea aboard research vessels. His subjects often were migratory fishes moving from tropical to temperate Atlantic coastal waters. The very nature of his responsibilities required cooperation and collaboration with many international fisheries organizations, for which he supervised and directed innumerable status reports. He served in varying capacities for many international groups, including the International Commission for Northwest Atlantic Fisheries (ICNAF), International Commission for Conservation of Atlantic Tunas (ICCAT), International Council for the Exploration of the Sea (ICES), Inter-American Tropical Tuna Commission (IATTC), and the Food and Agricultural Organization of the United Nations (FAO).

Noel Tibbo was a competent and productive scientist in his own right. He was sole or principal author in some 40 primary publications, mainly on Atlantic herring, first in Newfoundland and Labrador and later in the Maritimes. His work on Atlantic herring covered all aspects, from larvae through to the adult populations and commercial fisheries. However, his strength lay in his unique ability to organize and to work with others in the analysis and presentation of data. This is exemplified by an impressive list of collaborative scientific papers from investigations on Atlantic herring, but also on sharks and swordfish, which he supervised and directed, and innumerable status reports to different international fisheries organizations.

Noel retired from the Department of Fisheries and Oceans (now Fisheries and Oceans Canada) in 1975, but he pursued his interest in fisheries development from 1975 to 1978 by serving with the Canadian International Development Agency (CIDA) as a fisheries advisor consultant for projects in Senegal, Sri Lanka, Nicaragua, and Guyana, interspersed with 2 years (1976–1977) as manager of the Atlantic Herring Fishermen's Cooperative Ltd., a nice culmination of his lifelong involvement with herring. However, this did not spell the end of his active involvement with fisheries, for he then went on to found a consulting company, Fisheries and Marine Consulting Ltd., specializing in fisheries management and marine biology, in which he served as president, until finally really retiring.

Management and organization was a thread that ran through Noel Tibbo's life, in civic aspects as well as scientific. His talents were well-known and appreciated in the Town of St. Andrews, New Brunswick, where he served as Deputy Mayor, and on the District Board of School Trustees, as well as on various other committees. He took a particular pride in instituting the property-numbering system in the town. He was an enthusiastic curler, inducing all new scientists at the Biological Station to join the curling club, and was a keen golfer to the end of his life.

In a tribute to his death on 11 January 2003, it was stated that until his last day, Noel was mentally strong, his sense of humour and his determination intact. He is survived by his wife Edith (Pat), daughters Carla Gail (Gerry), Margaret Elizabeth (Peggy), Susan Lynn, son Robert Paul (Bob), and seven grandchildren.

J.S. Scott and W.B. Scott

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HOMMAGE

Simeon Noel Tibbo (1914–2003)

Simeon Noel Tibbo, un ichtyologiste bien connu et très estimé du Canada atlantique, est décédé le 11 janvier 2003 chez lui à Nanaimo (Colombie-Britannique).

Noel est né à Grand Bank (Terre-Neuve-et-Labrador) où il a effectué ses études primaires et secondaires. Il a poursuivi ses études supérieures à l'Université Memorial de Terre-Neuve (St. John's, Terre-Neuve-et-Labrador) et à l'Université Acadia (Wolfville, Nouvelle-Écosse), et a terminé son diplôme de sciences B.Sc. (Hons) en 1940. Sa carrière en ichtyologie a débuté avec un poste de chercheur adjoint au ministère des Ressources naturelles à St. John's (Terre-Neuve-et-Labrador), où il a participé à la recherche sur plusieurs espèces marines, dont le hareng atlantique (*Clupea harengus*) qui allait jouer un rôle primordial dans sa carrière. Au même moment, de 1943 à 1946, il a détenu une bourse d'études supérieures à l'Université de Toronto (Ontario) où il a obtenu un M.A.

La longue association de Noel avec St. Andrews (Nouveau-Brunswick), a commencé en 1946 avec sa nomination comme chercheur au sein du Comié d'étude du hareng atlantique (Atlantic Herring Investigation Committee) avec un intérêt particulier pour la biologie et la pêche du hareng atlantique dans le golfe du Saint-Laurent. Il a travaillé au sein de ce comité jusqu'en 1950 lorsqu'il a été associé à l'Office des recherches sur les pêcheries du Canada comme chercheur responsable de la section pélagique de la division des poissons marins. Le hareng atlantique était l'espèce d'intérêt primordial pour ce groupe qui se préoccupait de la biologie, de la structure des stocks et de la dynamique de population de l'espèce et qui devait formuler des recommandations pour sa gestion depuis le golfe du Saint-Laurent au golfe du Maine et à la baie de Fundy.

Durant les 20 premières années de sa carrière, Noel a produit au moins 35 articles scientifiques sur la biologie et la gestion du hareng atlantique. D'ailleurs, son intérêt pour le hareng atlantique et sa contribution à la connaissance de l'espèce se sont poursuivis pendant toute sa vie professionnelle. Au cours de ses premières recherches à Terre-Neuve et dont faisaient partie ses travaux de maîtrise, Tibbo est arrivé à la conclusion importante que, d'après le dénombrement des vertèbres et d'autres renseignements, les harengs des côtes sud et ouest de Terre-Neuve formaient des populations séparées et distinctes (S.N. Tibbo. 1956. J. Fish. Res. Board Can. **13**: 449–466). Ces conclusions ont plus tard été confirmées par des études de marquage et de recapture (J. Wheeler, Pêches et Océans Canada, Centre des pêches de

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l'Atlantique Nord-Ouest, Saint-John's (Terre-Neuve-et-Labrador), communication personnelle). Les travaux de Noel sur les différentes populations de hareng atlantique du golfe du Saint-Laurent (S.N. Messieh and S.N. Tibbo. 1971. J. Fish. Res. Board Can. **28**: 1009–1014) sont aussi à signaler, car ils posent les bases des évaluations de stock actuelles qui considèrent séparément les composantes à reproduction printanière et automnale (G. Chouinard, Pêches et Océans Canada, Centre des pêches du golfe, Moncton (Nouveau-Brunswick), communication personnelle). Ces contributions sont parmi les premiers exemples de l'identification de la biodiversité des stocks de poissons marins faite par les chercheurs canadiens.

Sous la direction de Noel Tibbo, la section pélagique a mené de nombreuses études sur la biologie et la pêche de poissons pélagiques tels que l'espadon (*Xiphias gladius*), le maquereau bleu (*Scomber scombrus*), les thons, particulièrement le thon rouge (*Thunnus thynnus*), et quelques espèces de requins. Les recherches sur les thons étaient d'importance car la pêche évoluait alors rapidement d'une pêche opportuniste au harpon à une pêche régulière à la palangre ciblée sur les espèces. Ces études couvraient la surveillance des prises commerciales et la biologie générale des espadons, en particulier leur répartition dans les eaux canadiennes, leur migration saisonnière, leur alimentation et leur reproduction. Noel a joué un rôle central dans ces travaux qui ont mené à sept publications additionnelles dont un Bulletin de l'Office des recherches sur les pêcheries fréquemment cité (S.N. Tibbo, L.R. Day, and W.F. Doucet. 1961. Fish. Res. Board Bull. No. 130) qui contient une synthèse de la biologie de l'espadon et de sa pêche au Canada.

Considérés dans leur ensemble, les travaux de Noel sont d'une envergure remarquable. En plus des recherches sur les harengs et les espadons, Noel a contribué à la compréhension de la faune diversifiée de poissons pélagiques des eaux atlantiques canadiennes, en particulier de la pastenague violette (*Pteroplatytrigon violacea*, antérieurement *Dasyatis violacea*; W.B. Scott and S.N. Tibbo. 1968. J. Fish. Res. Board Can. **25**: 1075–1076), de l'opah (*Lampris guttatus*; R.A. McKenzie and S.N. Tibbo. 1963. J. Fish. Res. Board Can. **20**: 1097–1099), de la coryphène commune (*Coryphaena hippurus*; S.N. Tibbo. 1962. J. Fish. Res. Board Can. **19**: 517–518), du requin obscur (*Carcharhinus obscurus*; S.N. Tibbo and R.A. McKenzie. 1963. J. Fish. Res. Board Can. **20**: 1101–1102), du mako à nageoires courtes (*Isurus oxyrinchus*; S.N. Tibbo, R.A. McKenzie, and W.B. Scott. 1963. J. Fish. Res. Board Can. **20**: 1343–1354), du requin océanique à longue nageoire (*Carcharhinus longimanus*; S.N. Tibbo. 1962. J. Fish. Res. Board Can. **19**: 517–518) et de la maraîche (*Lamna nasus*; R.A. McKenzie and S.N. Tibbo. 1964. J. Fish. Res. Board Can. **21**: 863–864).

Les responsabilités de Noel dans le domaine de la pêche pélagique l'ont souvent amené à travailler en mer sur les navires de recherche. Les espèces qu'il étudiait étaient fréquemment des poissons migrateurs qui se déplacent des eaux tropicales aux eaux côtières tempérées de l'Atlantique. La nature même de ses tâches exigeait une coopération et une collaboration avec plusieurs organismes internationaux des pêches, pour lesquelles il a supervisé et dirigé la rédaction d'innombrables rapports de situation. Il a participé à divers titres aux activités de nombreux groupes internationaux, dont la Commission internationale pour les pêcheries de l'Atlantique Nord-Ouest (CIPAN/ICNAF), la Commission internationale pour la conservation des thonidés de l'Atlantique (CICTA/ICCAT), le Conseil international de l'exploration de la mer (CIEM/ICES), la Commission interaméricaine tropicale des thonidés (CIATT/IATTC) et l'Organisation des Nations unies pour l'alimentation et l'agriculture (ONUAA/FAO).

Noel Tibbo était, dans son champ d'expertise, un chercheur compétent et productif. Il a été le seul ou le premier auteur d'environ 40 publications, principalement sur le hareng atlantique, d'abord à Terre-Neuve-et-Labrador puis dans les Provinces maritimes. Ses travaux sur le hareng atlantique couvrent l'ensemble du domaine, des populations larvaires aux adultes en passant par les pêches commerciales. Sa force particulière, cependant, était son habileté tout à fait remarquable d'organiser en collaboration avec les autres l'analyse et la présentation des données. Cette aptitude se reflète dans la liste impressionnante de travaux faits en collaboration sur les harengs atlantiques, mais aussi sur les requins et les espadons, qu'il a supervisés et dirigés, ainsi que les innombrables rapports de situation produits pour les différentes organisations internationales des pêches.

Noel a pris sa retraite du Ministère des Pêches et Océans (aujourd'hui Pêches et Océans Canada) en 1975, mais il a continué à s'intéresser au développement des pêches en agissant de 1975 à 1978 comme conseiller sur la pêche pour l'Agence canadienne de développement international (ACDI/CIDA) dans des projets au Sénégal, au Sri Lanka, au Nicaragua et en Guyane, tout en gérant l'Atlantic Herring Fishermen's Cooperative Ltd. pendant deux ans (1976–1977), un terme tout à fait approprié à une carrière consacrée à l'étude du hareng. Cela n'était pas pour autant la fin de son implication active dans les pêches, car il a ensuite fondé une compagnie de consultants, Fisheries and Marine Consulting Ltd., spécialisée dans la gestion des pêches et de la biologie marine dont il fut le président jusqu'à sa véritable retraite.

La gestion et l'organisation étaient les fils conducteurs des activités de Noel Tibbo tant dans le monde scientifique que municipal. Ses talents dans ces domaines étaient bien reconnus et appréciés. Il a servi de maire adjoint dans la ville de Saint-Andrews (New-Brunswick), et de membre du conseil des commissaires d'école du district ainsi que dans d'autres comités. Il était particulièrement fier d'avoir instauré le système de numérotation des propriétés de la ville. Il aimait beaucoup le curling et il incitait tous les nouveaux chercheurs arrivant à la Station de biologie de s'inscrire au club de curling; il fut aussi un golfeur enthousiaste jusqu'à sa mort.

Dans un hommage rendu à son décès le 11 janvier 2003, on a indiqué que jusqu'à son dernier jour, Noel a conservé intacts sa vivacité intellectuelle, son sens de l'humour et sa détermination. Il laisse dans le deuil son épouse Edith (Pat), ses filles Carla Gail (Gerry), Margaret Elizabeth (Peggy) et Susan Lynn, son fils Robert Paul (Bob) et ses sept petits-enfants.

J.S. Scott et W.B. Scott

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Flexible catch-balancing policies for multispecies individual fishery quotas

Daniel S. Holland and Guillermo E. Herrera

Abstract: In multispecies fisheries managed with individual fishery quotas, fishers targeting certain species often have insufficient quota to cover other jointly caught species. New Zealand employs a unique, dual quantity–price system to address this problem. In lieu of acquiring quota, fishers can opt to pay a fee per unit of catch known as deemed value (DV). Although designed primarily to create flexibility in catch balancing for individuals, this system can allow aggregate catches to exceed total allowable catches (TACs). The DV system reduces the likelihood that target species catches are constrained by TACs of bycatch species, but also increases the risk of overexploitation of bycatch species. Using a bioeconomic model, we evaluate the risk and efficiency of alternative DV policies in fisheries with one target and one bycatch species. Our simulations suggest that increasing DVs above ex-vessel price in response to TACs being exceeded can control risk of overexploitation without reducing overall efficiency; however, this does shift rents from owners of target species quota to owners of bycatch species quota.

Résumé : Dans les pêches commerciales multispécifiques gérées à l'aide de quotas de pêche individuels, les pêcheurs qui recherchent certaines espèces particulières n'ont souvent pas de quota suffisant pour couvrir les autres espèces capturées à la même occasion. La Nouvelle-Zélande utilise un système double particulier de quantité–prix pour résoudre le problème. Au lieu d'obtenir des quotas, les pêcheurs peuvent choisir de payer un tarif par unité de capture connu sous le nom de valeur réputée (DV). Bien que conçu principalement pour permettre aux pêcheurs individuels une flexibilité pour équilibrer leurs récoltes, le système peut aussi permettre à des récoltes combinées de dépasser les captures totales admissibles (TAC). Le système de DV réduit la probabilité que les captures d'espèces ciblées soient restreintes par les TAC des espèces accessoires, mais il augmente le risque de la surexploitation des espèces accessoires. Un modèle bioéconomique nous permet d'évaluer le risque et l'efficacité de différentes politiques de rechange de DVs dans des pêches avec une espèce cible et une espèce accessoire. Nos simulations indiquent que d'augmenter les valeurs réputées au-delà des anciens tarifs par bateau en réaction à un surpassement des TAC peut contrôler le risque de surexploitation sans diminuer l'efficacité globale, mais cela fait passer les loyers des propriétaires des quotas des espèces cibles aux propriétaires des quotas des espèces accessoires.

[Traduit par la Rédaction]

Introduction

Total allowable catches (TACs) in multispecies fisheries are typically set independently, with little or no consideration of relative catch rates and productivity. Consequently, catches are often out of balance with TACs. This can be particularly problematic when some stocks are substantially less productive than others. If it is not possible to reduce relative catch rates of less productive stocks, harvest of stronger stocks may have to be constrained below maximum sustainable yields (MSY) to protect weaker stocks (Ricker 1958; Paulik et al. 1967). This can dramatically reduce total yields and revenues from a multispecies complex, particularly if TACs are set to maintain all stocks at or above levels that support single-species MSY (Hilborn et al. 2004).

If relative catch rates can be altered through fishing behavior or gear choice, it may be possible to maintain higher TACs on productive stocks without putting weaker stocks at risk. However, in fisheries managed with competitively fished TACs (i.e., simple aggregate constraints on harvest), individual fishermen have no incentive to alter their catch composition if doing so increases their costs or decreases overall catch. Consequently, it is often necessary to close fisheries before some TACs are taken, so as to avoid overharvest and discarding of jointly caught species (OECD 1997).

Individual fishery quota (IFQ) systems provide economic incentives to reduce the imbalance of relative catch rates and TACs; scarcity of quota for a species increases the quota price and thereby reduces the net value (i.e., ex-vessel price less cost of quota) of harvesting the species. Fishers should

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therefore reduce harvest of species with the most constraining TACs (Squires et al. 1998), which mitigates the constraints weaker stocks impose on stronger ones. Branch et al. (2005, 2006) demonstrate that these incentives can significantly alter fishing behavior and the resulting catch composition.

Reducing the catch rates of one species may substantially increase the cost of taking jointly caught species, for example by necessitating the use of less efficient gear or operation in areas where catch rates of the target species are lower. These requirements can potentially reduce the value of the overall multispecies fishery, particularly if the species with the constraining TAC provides only a small portion of the overall value of the fishery. Costly avoidance of bycatch or some reduction in target species harvest may be desirable if higher catches of the bycatch stock put its viability at risk. In some cases, however, the TAC of the bycatch species is set unnecessarily low because of an underestimate of stock size, in which case exceeding the TAC may not necessarily threaten the viability of the stock. Such errors are likely for stocks that fluctuate substantially and also for stock assessments that are inaccurate and TAC adjustments that are not timely. In New Zealand, many bycatch stocks do not have formal assessments on which to base TACs, which are changed only rarely. In such cases, the implicit costs of a rigid TAC might be high.

When the assignment of TACs is inflexible because of information flows or logistics, and adjusting catch composition is costly, the overall value of a multispecies system may be increased by allowing fishers to exceed their quota holdings under certain conditions. This flexibility can also deter discarding, which is wasteful and can undermine the quality of data used in stock assessments. A number of mechanisms have been employed to add flexibility to catch balancing in IFQ systems: allowing exchange of quota across species; allowing fishers to carry back (borrow) or carry forward (bank) quota between years; charging a fee for catch in excess of quota holdings; and allowing fishers to surrender or discard catch they can not match with quota (Sanchirico et al. 2006). While these mechanisms are designed primarily to add flexibility to individual catch balancing, they can also lead to aggregate catches in excess of the TAC. This may increase the risk that some species will be depleted to the point where their viability is threatened. Ideally, a policy would add flexibility to a multispecies IFQ fishery without substantially increasing the risk of collapse of any component fish stock.

Although a variety of policies can facilitate catch balancing, our analysis focuses on the use of a deemed value (DV), a fee paid on each unit of catch in excess of quota holdings. This system is the primary means of enhancing catch-balancing flexibility in the New Zealand quota management system. DVs are set each year by the government, generally at a level lower than the ex-vessel price. This gives fishers an incentive to land (rather than discard) bycatch while at the same time reducing incentives to target species for which they have insufficient quota. DVs lower than ex-vessel prices are not, however, always sufficient to deter catches in excess of TACs. In fact, TACs for a number of species have been exceeded even with DVs set above ex-vessel prices.

A DV system effectively creates a dual quantity–price regime for managing catches at both the individual and aggregate level. In the presence of uncertainty about the size and productivity of fish stocks, price mechanisms have been shown to yield superior economic performance to quantity controls (Weitzman 2002). However, this result presumes a strong positive correlation between stock size and catch per unit effort (CPUE) and can be reversed when prices or harvest costs are uncertain (Hannesson and Kennedy 2005). Most previous analyses comparing price and quantity instruments consider single species fisheries; our analysis focuses on managing a bycatch species taken while targeting a more economically important species.

We use a model to explain how the biological and economic characteristics of multispecies fisheries affect the performance of different DV policies, both in terms of biological risk and economic efficiency. We also explore the impacts of these policies on both the distribution of rents among owners of quota for different stocks and the flow of DV payments to the government. Data constraints prevented us from developing predictive models of DV systems in specific multispecies fisheries. Rather, we investigate stylized two-species cases, each with one clear target and one clear bycatch species.

We explore two different cases that are based on actual New Zealand multispecies fisheries but abstract from some of their complexities. We examine fisheries whose biological characteristics suggest that flexible catch-balancing policies are particularly likely to pose risks. The first case includes two very slow-growing species with highly variable recruitment. The second contains a target species that is considerably more productive and variable than the bycatch species. The results of these two-species cases should provide insights relevant to more complex multispecies fisheries. However, because fisheries with three or more species may respond differently, the generality of our results may be limited.

Materials and methods

Model structure

We develop a bioeconomic model of an age-structured fishery consisting of a target species (1) and an incidentally caught (bycatch) species (2). We use the model to evaluate the economic performance and risk associated with different policies for setting TAC for each species and the DV charged by the government for catches of the bycatch species (DV_2) in excess of its TAC. In theory, fishers also have the option of violating TAC_1 for the target species and paying DV_1 for each additional unit of harvest; we make the simplifying assumption that the DV for the target is sufficiently high to discourage catch in excess of quota. This removes one choice variable on the part of the regulator and allows us to focus on the impact of the DV system on harvest of the bycatch species.

The biological dynamics of the model are described in Appendix A. Each species has a variable number of cohorts, depending on natural mortality. We employ stochastic Beverton and Holt stock–recruitment functions parameterized by virgin biomass B_0 and steepness h (mean recruitment as a fraction of virgin recruitment when spawning stock biomass,

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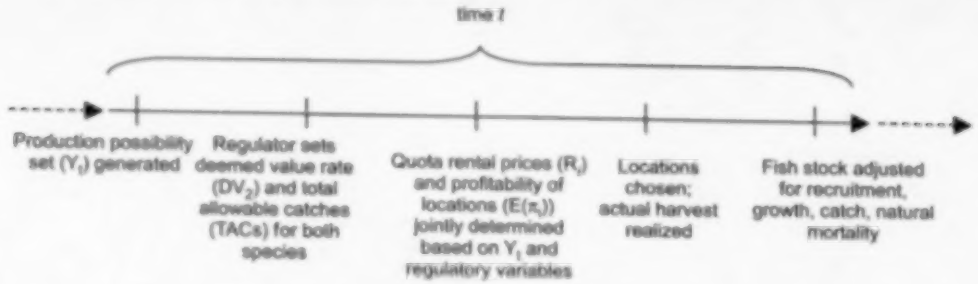
Fig. 1. Timeline of model steps within a representative time period.


time t
Production possibility set (Y_t) generated
Regulator sets deemed value rate (DV_2) and total allowable catches (TACs) for both species
Quota rental prices (R_t) and profitability of locations ($E(\pi_t)$) jointly determined based on Y_t and regulatory variables
Locations chosen; actual harvest realized
Fish stock adjusted for recruitment, growth, catch, natural mortality

SSB, is 20% of B_0). Our model, therefore, allows us to explore the effects on the risk of overexploitation of both stock–recruitment steepness and differences between selectivity and maturity ogives, both of which have been shown to be key risk factors (Francis 1992, 1993).

Multispecies fishery models are scarce (Butterworth and Punt 1999), and most assume exogenous relative catchability of species (e.g., Clark 1990; Overholtz et al. 1995; Holland and Maguire 2003). DV systems, however, presumably provide an economic incentive for fishers to adjust their catch mix, so it seems more realistic to model catch composition as endogenous. The present model depicts a harvest sector that maximizes expected profits by choosing from a set of discrete harvest opportunities in each time period, each opportunity yielding a different composition of harvest of the two species. Our model also endogenizes total catch, in particular that of the bycatch species. Both species are managed with TACs. However, if fishers find it profitable to do so, they may choose to exceed the TAC for the bycatch species and make DV payments to the government. Total effort and cost vary endogenously as a function of the collective location choices of the fleet.

The progression of model events in a representative time period is illustrated (Fig. 1). Once overall species abundance has been determined, the government chooses three regulatory parameters: TAC_j for each species $j = 1,2$; and DV_2, the deemed value for the bycatch species. The fleet then responds to these regulatory parameters by choosing where to fish. Note that we do not explicitly present an optimization problem on the part of the regulator. Instead, we compare the performance, along various dimensions, of different rules-of-thumb for choosing the regulatory parameters.

A stochastic set of discrete production possibilities is randomly generated each period. Depending on expected profits, the harvest sector (presumably a collection of profit-maximizing agents, though not modeled explicitly as such) makes a binary decision whether or not to exploit each discrete possibility. Profits depend upon expected catches in each location, ex-vessel prices, equilibrium quota rental prices emerging in each time period as described below, and DV_2.

Expected catches are a function of total recruited biomass of the stocks and an underlying covariance matrix **COV** — a function of the spatial distribution of the stocks and perhaps other factors affecting relative catch rates. The populations of the two stocks are distributed across S locations as represented by the $S \times 2$ matrix:

$$\text{(1)} \qquad \mathbf{Y} = \exp[\mathbf{VL} + \log(\overline{\mathbf{X}})]$$

V is an $S \times 2$ matrix of random numbers with zero mean and unit variance. **L** is the Cholesky factorization of **COV** (i.e., an upper triangular matrix satisfying $\mathbf{COV} = L'L$). Calculating the covariance of log(**Y**) generated in the simulations will, on average, recover the covariance matrix **COV** used to generate **L**. In our simulations, **COV** is hypothetical, but it could be calculated if there were adequate survey data covering the viable fishing grounds. A covariance matrix estimated from commercial catch would differ from **COV**, because harvesters' choices are biased toward more profitable locations.

COV does not change with the total abundance or mean catch of the two species; we implicitly assume that stocks redistribute themselves after each fishing season so as to restore the given relative spatial distribution. It is reasonable to assume that fishing effort may change the relative distribution and covariance of the species from that arising without fishing, but if the fishery has stabilized somewhat, this would be reflected in the survey data and thus the estimate of covariance. Recruits are spread evenly among locations, so $\overline{\mathbf{X}}$ is an $S \times 2$ matrix with all elements of each column $j = 1,2$ equal to total recruited biomass for species j divided by the number of locations S.

The production possibilities set itself is the $S \times 2$ matrix $q\mathbf{Y}$, the product of catchability coefficient (q) and the stock distribution (**Y**) from eq. 1. Simulated production possibility sets associated with different **COV** matrices are shown (Fig. 2). Each point in each panel represents a discrete production possibility (i.e., the combination of catches resulting from the exertion of a single unit of effort in a particular location). As the variance terms in **COV** are increased, the variability in catch rates across locations for each species increases. As the covariance terms in **COV** are increased relative to the variance terms, the correlation of species catches increases, and fishers have fewer options that provide high catches of one species and low catches of the other.

We employ a reduced-form representation of the behavior of the fleet in aggregate during each time period. While this prevents us from drawing conclusions about decision making at the trip or vessel level in response to the regulatory framework, it simplifies our analysis and computations and allows us to focus on system-wide effects of different management approaches. The fleet chooses to exploit a subset of the available production choices, based on expected catches associated with each choice and the net prices received for

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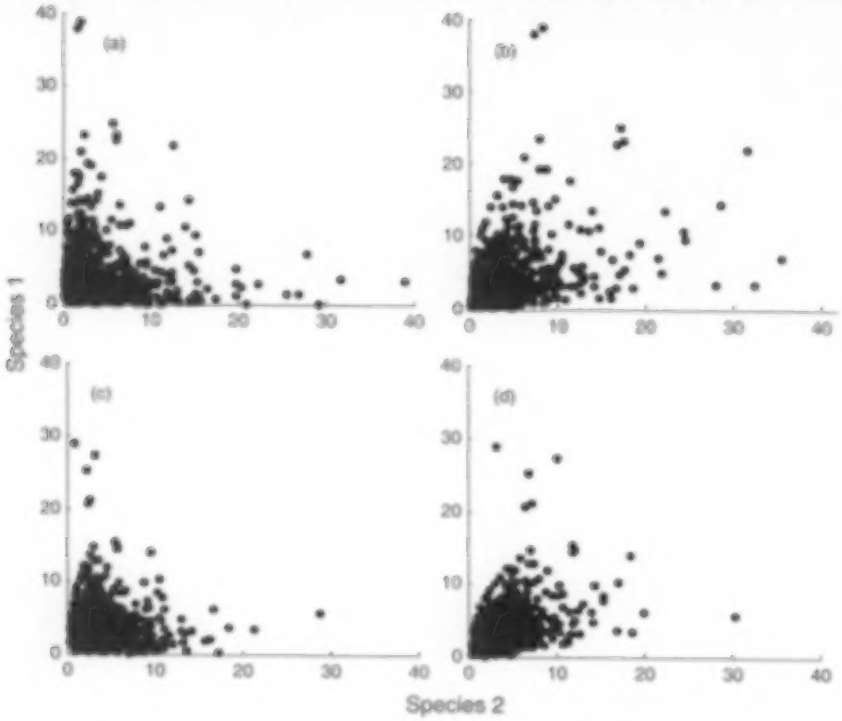
Fig. 2. Scatterplot of a production possibility set of discrete catch opportunities across the 1000 production choices simulated assuming recruited stock biomass of 10 000 for both species, catchability of 0.35, and four different values of the covariance matrix: (*a*) variance 1.0 and covariance 0; (*b*) variance 1.0 and covariance 0.5; (*c*) variance 0.5 and covariance 0; and (*d*) variance 0.5 and covariance 0.25.



each species. Locations i are ranked in order of descending expected profits, π_i, calculated as

$$(2)\quad \pi_i = (P_1 - R_1)\, q_1 Y_{i,1} \exp(\sigma_1^{\varepsilon} \varepsilon_{i,1}^{\varepsilon}) + (P_2 - R_2)\, q_2 Y_{i,2} \exp(\sigma_2^{\varepsilon} \varepsilon_{i,2}^{\varepsilon}) - C, \quad \varepsilon_{i,j}^{\varepsilon} \sim N(0,1)$$

P_1 and P_2 are exogenous ex-vessel prices (paid to fishermen at the dock) for target and bycatch, respectively; R_j are the rental prices for species j quota, determined as described below; $Y_{i,j}$ are biomasses of species j at location i; and q is assumed equal across species and locations. The cost C associated with harvesting a location is assumed to be constant across the options. Relative profitability includes the cost of renting quota, so profits depend upon net prices ($P_j - R_j$) received for each species rather than just ex-vessel prices.

$\varepsilon_{i,j}$ are location-specific random variables with zero mean and unit variance. σ_j^{ε} are multipliers on errors in fishers' expectations of profits in each location, because of uncertainty about either stock abundances or the equilibrium quota prices. Actual catch is deterministic once the location is chosen. We initially assume perfect information ($\sigma_1^{\varepsilon} = \sigma_2^{\varepsilon} = 0$), and then later explore the implications of relaxing this assumption.

Since 2001 in New Zealand, quota shares have generated annual catch entitlements (ACE): catch rights denominated in kilograms that must be used during a given fishing year. Catches are now balanced with ACE rather than quota per se. ACE replaced quota leasing and other forms of short-term contracting between owners and users of quota. The R_j in our model is effectively quota rental prices for species j = 1,2, but we refer to them henceforth as ACE prices. R_j are key determinants of relative profitability of different locations and therefore of the location choices and realized aggregate catches. These prices are endogenously determined as functions of the regulatory parameters and stock distributions.

The ACE price for the bycatch species, R_2, equilibrates at the highest level that does not constrain catches of the bycatch species below the TAC, with the additional stipulation that $R_2 \leq DV_2$. Paying DV_2 is a perfect substitute for purchasing ACE for species 2, so fishers will not lease quota for a price higher than DV_2.

The algorithm used to determine the ACE price R_2 for the bycatch species therefore starts by setting $R_2 = DV_2$. If the aggregate catch of species 2, H_2, exceeds TAC_2 at this price, then the equilibrium remains $R_2 = DV_2$; catch in excess of TAC_2 results in DV payments of $DV_2(H_2 - TAC_2)$ to the government (as illustrated in Fig. 3). If $H_2 < TAC_2$ when $R_2 = DV_2$, R_2 is progressively lowered, and the new aggregate catches are computed. The process is repeated as long as $H_2 < TAC_2$, although R_2 also has a lower bound of 5% of the bycatch ex-vessel value. That is, $R_2 \geq 0.05P_2$, even if $H_2 < TAC_2$ at that price. This lower bound was chosen arbitrarily, based on the presumption it would be necessary to offset cost recovery levies and other transactions costs.

In theory, R_1 should equilibrate on a price that drives marginal profit to zero (i.e., $\pi_i = 0$ in the last location fished)

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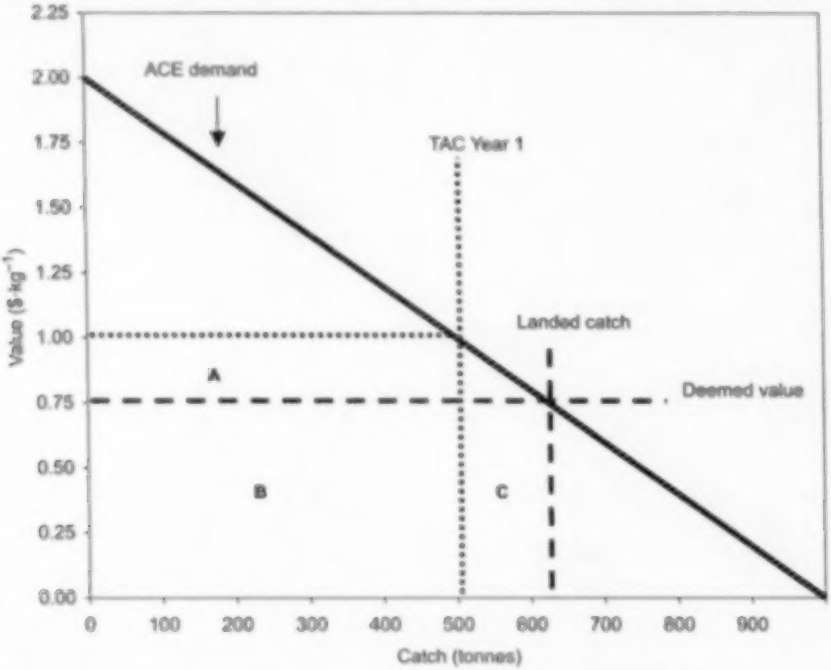
Fig. 3. Derived demand and equilibrium annual catch entitlement (ACE) prices for a species taken as bycatch. Derived demand for ACE for the bycatch species is downward sloping, since reducing bycatch becomes progressively more difficult and costly as the total bycatch is reduced. Supply of ACE is fixed at the total allowable catch (TAC), but catch can exceed the TAC if fishers pay deemed value on excess catches. In this example, the deemed value is set below the ACE price that would have equilibrated ACE demand with the TAC. Deemed value acts as a ceiling on ACE price, and catch expands beyond the TAC to the intersection of ACE demand and deemed value. Quota owners receive area B in ACE revenues, and the government receives area C in deemed value revenues. Had the deemed value been higher than $1.00, ACE price would have equaled $1.00. Catch would have been constrained to the TAC, and quota owners would have garnered rents equal to areas A plus B while deemed value revenues would have been zero.



given the R_2 is determined as described above. Our method approximates this market clearing level of R_1 while avoiding a marginal price overly influenced by the catch ratio of the last single catch event. R_1 is calculated such that the average residual profit per kilogram of target catch in the least profitable 5% of areas fished is zero. Therefore

$$(3)\qquad R_1 = \left[\sum_{i=E-0.05E}^{E} P_1 q_1 Y_{i,1} \exp(\sigma_1^2 \varepsilon_{i,1}^2) + (P_2 - R_2) q_2 Y_{i,2} \exp(\sigma_2^2 \varepsilon_{i,2}^2) - C \right] \Big/ \left(\sum_{i=E-0.05E}^{E} q_1 Y_{i,1} \right)$$

where the E fished locations are ranked from highest to lowest by π_i from eq. 2, such that $I = 1$ for the most profitable location and $I = E$ for the last location fished that year. Note that in the remainder of fished locations ($I > 0.95E$), π_i would on average be greater than zero, creating inframarginal profits after ACE payments and harvests costs are deducted from gross revenues.

In short, ACE prices for the two species depend upon the exogenous and perfectly inelastic supply of ACE (the TACs), the derived demand for ACE, and DV_2, which serves as a backstop. Determination of the endogenous ACE prices R_i by the algorithm described above is dual to the problem of determining the optimal production levels of two jointly produced goods, given a set of exogenous prices and a production possibility frontier, as depicted in Turner (1997). Rather than finding the point on the production possibility frontier that is tangent to an exogenous price ratio, our model finds the ACE prices R_1 and R_2, such that a line with slope $-(P_1 - R_1)/(P_2 - R_2)$ is tangent to the point on the production possibility frontier exogenously determined by the TACs. Effectively, it determines a set of net prices that leads to a combination of aggregate catch levels that satisfies the TACs and also maximizes profits. Our scenario is complicated slightly by the fact that the TACs do not constitute an inviolable constraint; fishers can (in aggregate) choose to expand production along the species 2 dimension beyond that dictated by TAC_2, at a price (i.e., by paying DV_2 on catches above TAC_2).

Another feature of our analysis is that contrary to the situation pictured in Turner (1997), our simulations presume a discrete set of harvest options. Aggregate catch is determined by summing the catches that result from the chosen locations. These choices do not produce a smooth aggregate production possibility frontier.

How locations are chosen from a given production possibility set is visually illustrated (Figs. 4*a*–4*d*). A full representative set of harvest options is shown (Fig. 4*a*), as well as

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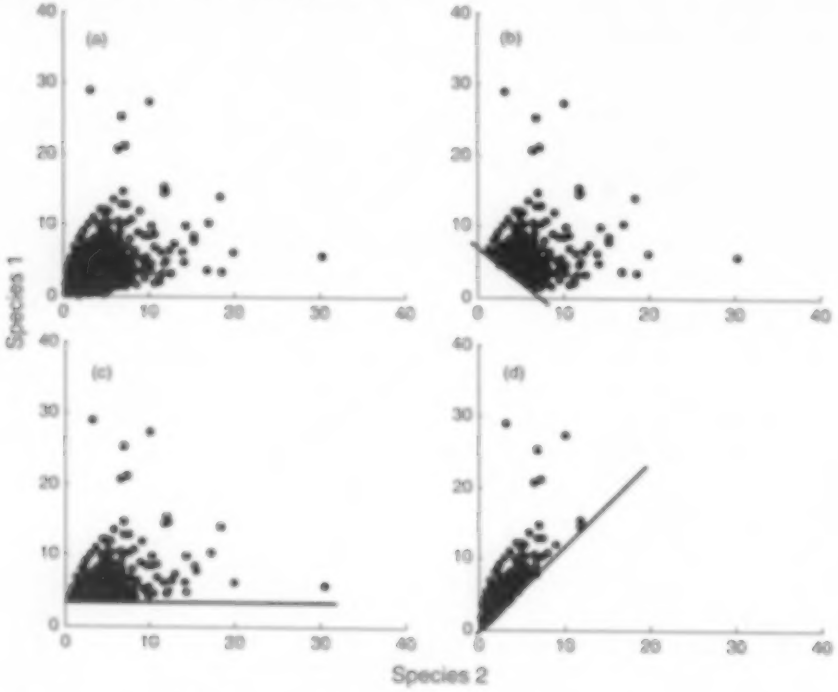
Fig. 4. Scatterplot of (*a*) production possibility set of discrete catch opportunities across the 1000 simulated production choices assuming recruited stock biomass of 10 000 for both species, catchability of 0.35, and a covariance matrix with variance of 0.5 for both species and covariance of 0.25. Panels *b* through *d* show the sets of profit-maximizing production choices consistent with catches exactly exhausting both total allowable catches (TACs), where the TAC of species 1 is 2000 and the TAC of species 2 is reduced from (*b*) 2000 to (*c*) 1500 to (*d*) 1000. Choices below the net price ratio line (solid lines in panels *b*–*d*) are eliminated, since they are less profitable than the remaining choices, which collectively exhaust both TACs.



the set of locations that maximizes total fishery rents subject to the binding TACs (Figs. 4*b*–4*d*). To simplify the example, we assume that DV is set sufficiently high to deter violations of either TAC; we also assume that harvest costs are sufficiently low so it is profit-maximizing to fully exploit both TACs. The TAC for species 2 is progressively decreased moving from Fig. 4*b* through Fig. 4*d*. Fishers exploit only the most profitable subset of options, based on catch rates and net prices according to eq. 2. The resulting production set consists of the locations with catch compositions above the net price ratio line (NPRL), where the slope of NPRL is

$$(4) \qquad m_{\mathrm{NPRL}} = -\frac{P_2 - R_2}{P_1 - R_1}$$

Choices shown in Fig. 4*a* that are below NPRL (the solid lines shown in Figs. 4*b*–4*d*) are dropped from the production choice set; they are technically feasible but not part of the constrained profit-maximizing solution of fishers.

As the TAC for Species 2 is lowered from 2000 (Fig. 4*b*) to 1000 (Fig. 4*d*), a lower net price for species 2 is required to deter fishers from exhausting the TAC of species 2 before taking the TAC of species 1. NPRL goes from having a negative slope (Fig. 4*b*, where both net prices are positive) to a positive slope (Fig. 4*d*, where the net prices of species 2 is negative), causing the fleet to choose locations that avoid high catches of species 2. A negative net price for species 2, where $R_2 > P_2$, is required to give NPRL a positive slope (as in Fig. 4*d*) and constrain catches to the lower TAC. If R_2 were constrained to be less than or equal to P_2 (e.g., by a DV equal to P_2), NPRL could not have a positive slope and catches of species 2 would exceed the TAC.

Although the covariance matrix that determines the relative catch rates across location choices is constant, actual catch rates vary stochastically from one year to the next. At the beginning of each year, a new matrix of random numbers is generated and the fish populations are remixed. We do not attempt to model how the spatial distribution would develop over time in response to fishing and fish movement, and we assume that harvest costs are homogeneous across the production choice set. To do otherwise would greatly complicate the model with little analytical or conceptual benefit.

Policy evaluations

Our analysis focuses on cases where one species is targeted and the other taken primarily as bycatch. These are the most problematic cases, because even DVs set above the ex-vessel price of bycatch may not always provide sufficient incentives to discourage catching it — in particular if reducing bycatch results in a major drop in target species catch rates. This problem is exacerbated when the ratio of bycatch to target species catch is very small, in which case very large pen-

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alties per unit of bycatch may be needed to change targeting behavior.

Trade-offs in economic efficiency and biological risk associated with alternative DV and harvest strategies depend on a number of biological and economic factors. These include, but are not limited to, the following: relative potential size of target and bycatch stocks (e.g., B_0); productivity factors (e.g., steepness and variability of recruitment, individual growth rates, and natural mortality); spatial correlation of the two species, which affects the ability of the fleet to alter relative catch rates through fishing choices; and ex-vessel prices and harvest costs. A complete factorial analysis of all possible combinations of these characteristics is beyond the scope of this analysis. Instead, we focus on a few cases with characteristics that exacerbate the risk that the bycatch stock will be overexploited. We do not attempt to represent the full complexity of multispecies fisheries, but rather model pairs of species that are consistent with known characteristics of actual New Zealand multispecies fisheries.

The first case has orange roughy (*Hoplostethus atlanticus*) as the target species and smooth oreo (*Pseudocyttus maculatus*) as the bycatch. Biological parameters are based on the mideast coast stock of orange roughy (ORH3A) and the Chatham Rise smooth oreo stock (OEO3A) (Table 1). Although the two stocks are similar in size and productivity, the price of orange roughy ($3.70) is nearly five times the price of smooth oreo ($0.80), so targeting decisions are largely driven by catch rates for orange roughy. This case is illustrative of the risk–efficiency trade-offs in a fishery with two jointly caught species that are both very long-lived and slow growing, with highly variable recruitment. The target exploitation rate for orange roughy is also higher than that for smooth oreo, increasing the risk that smooth oreo will be overexploited.

The second case has red cod (*Pseudophycis bachus*) as the target species and elephantfish (*Callorhinchus milii*) as the bycatch. This case is representative of the red cod fishery off the east coast of the South Island in New Zealand in QMA 3, which includes the red cod stock RCO3 and the elephantfish stock ELE3. The biological parameters of the model are based on these stocks (Table 1). Although the price of red cod ($0.54) is only about one-third that of elephantfish ($1.58), the targeting decisions for these simulations are dominated by red cod catch rates because of the high ratio of red cod to elephantfish in catches. The MSY for RCO3 is more than an order of magnitude higher than that for ELE3. This case is of particular interest because the bycatch species, elephantfish, is considerably less productive than the target species, red cod, so its viability might be threatened if exploited at rates appropriate for the target stock.

In the base cases for both scenarios, we assume a relatively high spatial covariance of species (0.5). High covariance increases both the opportunity costs of avoiding bycatch and the potential risk of overexploiting the bycatch stock. Results from models with high covariance are expected to be more conservative; risk and efficiency losses are likely to be lower if covariance is lower. As a sensitivity analysis, we also run simulations where the covariance is reduced to 0.25 and 0.0.

Consistent with prescriptions of the New Zealand Fishery Act 1996 (Part 3, No. 13, s. 2(a)), TACs in the base cases are set to maintain SSB at or above levels that support MSY in the case of deterministic recruitment. For Case 1, the base case simulation adjusts TACs of both orange roughy and smooth oreo annually to achieve a constant fishing mortality equal to the deterministic F_{MSY}. The high variability of red cod makes it impractical to take this approach. In practice, the TAC for red cod has been constant at 12 389 tonnes since 1992, and between 12 000 and 13 000 tonnes since introduction to the New Zealand quota management system in 1986. Therefore, for Case 2, the base case simulation assumes the TAC for red cod is fixed at the deterministic MSY (12 529), while the TAC for elephantfish is adjusted annually to maintain constant fishing mortality F_{MSY}. As a sensitivity analysis, we also simulate alternative TAC-setting policies for both cases, as discussed in the results.

Historically, DVs have often been set at some fraction of ex-vessel price and are mostly kept below ex-vessel prices to encourage fishers to land catches rather than discard them (a policy consideration, despite the illegality of discarding). This is not entirely consistent with the mandate of the New Zealand Fishery Act 1996, which states that the primary consideration when setting DVs should be the incentive to balance catch with ACE. Because DV is a perfect substitute for ACE, DVs must be maintained above ACE prices to create incentives for individuals to purchase ACE rather than simply paying DVs. The simulations set the DV for the bycatch species at 150% of the prior year's ACE price. However, the 1-year lag between the DV and the ACE price means that the DV in a given year of the simulation can be higher or lower than 150% of that year's ACE price. If demand for ACE to cover bycatch decreases, the ACE price falls and the DV is reduced the next year; the opposite occurs when ACE demand rises. In all simulations, the DV for the bycatch species is adjusted each year to be equal to 150% of the prior year's ACE price, subject to constraints on minimum and maximum levels.

DVs are constrained between a minimum of 50% of the ex-vessel price and a maximum equal to either the ex-vessel price or twice the ex-vessel price. Note that even a DV equal to twice the ex-vessel price does not necessarily create sufficient incentive to constrain bycatch within the TAC; the opportunity cost of avoiding a unit of bycatch (e.g., forgone profits from harvesting the target species), rather than the value of the bycatch itself, is what determines whether it is worth paying DV. This assumes sufficient enforcement to deter illegal discarding.

The model does not explicitly set a DV for the target species. In reality, fishers can balance catch of target species with DV just as they can bycatch; thus catch of the target species could exceed the TAC. Our implicit assumption is that DVs are utilized only as a tool to allow flexibility in catch balancing for the bycatch species. Thus the DV for the target species is always kept high enough to remove any profits from targeting the species without ACE, so the TAC for the target is never exceeded.

Since we assume that recruitment and other parameters vary stochastically, we evaluate average results for each simulation from 500 replications, each of which runs for 100 years, starting with randomly generated starting stocks consistent with the target fishing mortality rates. We compare the performance of alternative maximum DVs in terms of risk, eco-

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Table 1. Simulation parameters for two fishery cases, each with two species.

		Case 1		Case 2	
Parameter	Description	Orange roughy (*Hoplostethus atlanticus*)	Smooth oreo (*Pseudocyttus maculatus*)	Red cod (*Pseudophycis bachus*)	Elephantfish (*Callorhinchus milii*)
J	Number of year classes	80	80	13	13
B_0	Unfished spawning stock biomass (tonnes)	100 000	85 000	60 000	7 000
h	Steepness of stock recruitment function	0.75	0.75	0.9	0.75
σ_r^2	Standard deviation of recruitment residuals	1.1	0.65	1.5	0.4
M	Natural mortality	0.045	0.063	0.75	0.35
L_∞	von Bertalanffy maximum length	37.2	50.8	72.5	94.1
K	von Bertalanffy K (growth coefficient)	0.065	0.047	0.44	0.345
t_{zero}	von Bertalanffy t_{zero}	–0.5	–2.9	0.015	–0.535
a	Length–weight conversion: weight = a(length)^b	0.0921	0.029	0.010 95	0.0091
b	Length–weight conversion: weight = a(length)^b	2.71	2.9	2.9755	3.02
S^1	Age where selectivity equals 1	55	21	3	5
S^L	Years before S^1 where selectivity equals 0.5	12	0.5	1	3
A^1	Age where maturity equals 1	55	31	3	5
A^L	Years before A^1 where maturity equals 0.5	12	1.5	1	1
F_{targ}	Fully recruited target F for base model	0.13	0.06	0.98	0.31
TAC_{targ}	Target fixed total allowable catch (tonnes)	2 657	2 302	12 529	825
q	Catchability coefficient	0.15	0.15	0.33	0.33
P	Ex-vessel price	3.70	0.80	0.54	1.58
C	Harvest cost per harvest event	10 000	na	6 250	na

Note: na, not applicable.

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nomic efficiency, and the distribution of fishery rents between target and bycatch quota owners.

The base case simulations assume fishers have perfect information about the relative profitability of different locations, thus the uncertainty multipliers σ_1^2 and σ_2^2 in eq. 2 are set to zero. Under this assumption, the most profitable locations are fished. We then explore the impacts of introducing uncertainty regarding relative profitabilities of locations by setting both σ_1^2 and σ_2^2 to 0.25. This leads to a lognormal distribution of perceived net revenues for each species. This distribution has a mode equal to the true value but is skewed, with the perceived value falling between 50% and 200% of the true value approximately 95% of the time.

We also evaluate the impacts of introducing uncertainty in the stock assessment used in the setting of TACs. This uncertainty is introduced as a random multiplicative observation error in the estimate of recruited stock biomass (RSB):

$$\text{(5)} \qquad \text{RSB}_{\text{observed}} = \text{RSB}_{\text{actual}} \exp(\varepsilon^{\text{ar1}})$$

where ε^{ar1} is an autocorrelated, normally distributed random variable with mean zero, a standard deviation of 0.25, and first order autocorrelation of 0.40. With this autocorrelation, stock assessment error is likely to persist for a few time periods, but then dissipate.

The model tracks a variety of different performance indicators: average catches, total fishery rents and their distribution, and risks of overexploitation. Several possible measures of risk might be used to evaluate policies. Hilborn (1997) argues that measures of risk beyond the average level and variability of catches and rents may be unnecessary if models include the mechanisms that create variability in productivity (e.g., variation in recruitment, natural mortality, and fishing mortality). However, some potential biological risks associated with low stock levels are not modeled in these simulations (e.g., depensation). Therefore, we track and report proxies for biological risk based on biomass reference points.

Reference points based on SSB are commonly used by fishery managers to evaluate the riskiness of alternative harvest strategies. Target or limit reference points for SSB set at some percentage of B_0 are often used (Butterworth and Punt 1999). However, the risk of collapse when stocks fall below these levels depends greatly on the productivity of the stocks, and B_0 is notoriously difficult to estimate (Myers et al. 1994). Myers et al. (1994) recommend recruitment-related biomass reference point (50% R_{max}) equal to the SSB that produces 50% of the maximum recruitment from a fitted stock–recruitment relationship. Rather than choose a single measure of risk, we report the percentage of years that SSB falls below a variety of SSB reference points including 10% of B_0 and 50% R_{max}. We also monitor the frequency with which SSB falls below 20% B_0, but do not use this as a risk threshold, since several of the stocks naturally fluctuate around this level under an F_{MSY} policy without any TAC violations. Note that the SSB of either species can fall below the given biological reference points without catches exceeding TACs. Our primary interest here is to determine if the frequency with which these reference points are breached is decreased as a result of stricter DV policies (i.e., higher maximum DVs) that reduce the frequency or magnitude of TAC overruns.

Overall economic efficiency is represented by the average annual revenues across both species minus harvest costs. The simulations also track the distribution of fishery rents. This includes the share of profits accruing as ACE revenues to quota owners of the target and bycatch species, DV payments accruing to the government, and residual, inframarginal rents accruing to fishers. The simulations determine ACE prices for both the target and bycatch species endogenously. For the target species, ACE revenues for quota owners are equal to the total catch of the target species multiplied by the ACE price. The same is true for the bycatch species, except that DVs are paid on any catch exceeding the TAC, and those revenues are assumed to accrue to the government. Because catch rates at different fishing locations vary but ACE prices are constant, some locations generate inframarginal profits once harvest costs and ACE costs are deducted from revenues. These latter profits presumably accrue to fishers who may or may not be quota owners.

Results

Case 1: biological risk and stock productivity

The incidentally caught species (smooth oreo) for this case is vulnerable to overexploitation because the fish recruit to the fishery 10 years before maturity. The SSB of smooth oreo falls below 10% of B_0 and below 50% R_{max} a substantial proportion of the time for some simulations (Table 2). This is generally not the case for simulations where both TACs are adjusted to maintain F_{MSY}, unless covariance of the stock distributions is high. For the base case scenario, with TACs for both species set at F_{MSY} and a high covariance (0.5) of species distribution, the SSB of smooth oreo falls below 10% of B_0 29% of the time and below 50% R_{max} 4% of the time when the maximum DV is $0.80. However, the frequency of breaching either reference point falls to less than 1% with the higher maximum DV of $1.60. For covariance of zero or 0.25, SSB of smooth oreo never falls below 10% of B_0 or 50% R_{max}, even with the lower maximum DV.

We also simulate a policy where the TAC for orange roughy is set to maintain fishing mortality at 65% of F_{MSY}. This TAC policy, which we refer to as the maximum economic yield (MEY), yields the highest total annual rents from the orange roughy – smooth oreo fishery when recruitment is deterministic. It is not a true MEY policy, since it does not account for the dynamic nature of the fishery or variability, focusing solely on equilibrium rents in the deterministic case. A 35% reduction in the target fishing mortality for orange roughy completely prevents smooth oreo from ever falling below the biological reference points, even with the highest level of covariance and the lowest maximum DV. This is achieved with less than a 5% reduction in average catch of either orange roughy or smooth oreo.

Adding uncertainty in the relative profitability of locations ($\sigma_1^2 = \sigma_2^2 = 0.25$) to the base case scenario slightly increases the frequency that SSB falls below reference points. Relative to the base case scenario with a maximum DV of $0.80, the frequency of SSB falling below 10% of B_0 increases from 29% to 32%, and the frequency of SSB falling below 50%

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Table 2. Frequency (average percentage of years) of breaching biomass reference points for smooth oreo (*Pseudocyttus maculatus*) in Case 1.

Simulation variations			Percentage of years SSB < 10% B_0		Percentage of years SSB < 50% R_{max}	
TAC policy	Covariance	Other	Max DV = \$0.80	Max DV = \$1.60	Max DV = \$0.80	Max DV = \$1.60
FF–FF	0.5		29	0	4	0
FF–FF	0.25		0	0	0	0
FF–FF	0		0	0	0	0
FF–FF	0.5	MEY	0	0	0	0
FF–FF	0.5	UFD	32	0	5	0
FF–FF	0.5	USA	24	1	5	0
FF–FF	0.5	$q \times 2$	93	45	91	35
FF–FT	0.5		46	15	25	7

Note: SSB, spawning stock biomass; MSY, maximum sustainable yield; DV, deemed value charged per kilogram; FF, constant fishing mortality set at F_{MSY} except in MEY simulation; FT, fixed total allowable catch (TAC) set at deterministic MSY unless otherwise specified; MEY, maximum economic yield; UFD, uncertainty in fish distribution; USA, uncertainty in stock assessment; $q \times 2$, catchability coefficient for the bycatch species is doubled.

R_{max} increases from 4% to 5%. But results with higher DVs are unchanged from the base case with no uncertainty in relative profitability.

Introducing uncertainty in the stock assessment for orange roughy has variable impacts on the percentage of years that smooth oreo SSB falls below reference points. For the base case with an F_{MSY} policy for both stocks, a high covariance, and a DV of \$0.80, the frequency of SSB falling below 10% of B_0 decreases from 29% to 24% of years when stock assessment error is added. With a maximum DV of \$1.60, SSB falls below 10% of B_0 less than 1% of the time.

As an additional sensitivity analysis, we consider a scenario where the catchability coefficient on smooth oreo is assumed to be twice that of orange roughy. This makes it harder and more costly to avoid bycatch and increases the frequency of biomass reference points being breached. With covariance of 0.5, SSB falls below 10% of B_0 93% of years with a maximum DV of \$0.80 and 45% of years with a maximum DV of \$1.60. SSB falls below 50% R_{max} 91% of years with a maximum DV of \$0.80 and 35% of years when the maximum DV is \$1.60. Allowing the DV to increase to a maximum of \$3.20 (four times the ex-vessel price), however, does eliminate any breaching of the biomass reference points.

In practice, the TAC for smooth oreo is not adjusted annually to maintain a constant fishing mortality, though it has been reduced over time as the stock has been fished down. As another sensitivity analysis, we consider scenarios where the smooth oreo TAC is fixed at the deterministic MSY while the orange roughy TAC is adjusted to maintain F_{MSY}. For this TAC policy, smooth oreo SSB falls below reference points considerably more frequently than when both TACs are adjusted to F_{MSY}. With covariance of 0.5, SSB falls below 10% of B_0 46% of years with a maximum DV of \$0.80 and 15% of years with a maximum DV of \$1.60. SSB falls below 50% R_{max} 25% of years with a maximum DV of \$0.80 and 7% of years when the maximum DV is \$1.60.

Although our focus is on the effect DV policies have on biological risk for the bycatch species, we also track SSB relative to reference points for the target species. SSB stays above all three reference points more than 99% of years for all scenarios modeled. Orange roughy catches decline below the TAC when the stock declines because taking the full TAC becomes unprofitable as catch rates fall. This reflects our implicit assumption that the relative distribution of the stock remains constant as the stock contracts, so CPUE falls as the stock declines.

Case 1: economic efficiency and distribution of resource rents

For the base case model (TACs set at F_{MSY} for both stocks and the highest covariance of 0.5), total annual average fishery rents are \$8.23 million and increase slightly to \$8.34 million when the maximum DV is \$1.60 (Table 3*a*, Fig. 5). Although total rents are not greatly affected by the maximum DV, the distribution of rents is. The share of total fishery rents accruing to orange roughy ACE owners is 31% with a maximum DV of \$0.80, but falls to 10% when the maximum DV is raised to \$1.60. In contrast, total ACE revenues for smooth oreo rise from 15% to 37% of total fishery rents when the maximum DV is raised. DV payments actually decline from 9% of total rents to 4% with the higher maximum DV, reflecting the fact that doubling the maximum DV reduces catches above the TAC by over 50% on average. Inframarginal profits increase from 46% of total rents to 49% when the DV is raised to \$1.60. In sum, there are actually small gains in total fishery rents with the stricter DV policy for this scenario, but it results in substantial shifts in fishery rents from orange roughy ACE owners to smooth oreo ACE owners.

If covariance is 0.25 and the maximum DV is set at \$0.80, there is no difference in the total fishery rents or in the share of fishery rents accruing to orange roughy ACE owners (Table 3*a*) relative to the base case with higher covariance. However, smooth oreo ACE revenues increase while DV revenues fall. As with the base case, increasing the maximum DV reduces orange roughy ACE revenues and DV payments while increasing smooth oreo ACE revenues and inframarginal profits. With zero covariance, total fishery rents are slightly lower than in the base case (\$8.14 million). The share of rents earned by orange roughy ACE owners is similar to the base case, but a higher percentage of rents accrue to smooth oreo ACE and a smaller share to DV payments. With zero covariance, increasing the maximum DV has little impact on either the total or the distribution of rents because the lower DV is sufficient to keep smooth oreo catches within the TAC.

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Table 3. Average annual fishery rents and distribution of rents as percentage of total for (*a*) Case 1 and (*b*) Case 2.

(*a*) Case 1.

Simulation variations			ACE revenues (%)									
			Orange roughy (*Hoplostethus atlanticus*)		Smooth oreo (*Pseudocyttus maculatus*)		Deemed values on smooth oreo (%)		Inframarginal profit (%)		Combined annual fishery rents (millions of dollars)	
TAC policy	Covariance	Other	DV $0.80	DV $1.60	DV $0.80	DV $1.60	DV $0.80	DV $1.60	DV $0.80	DV $1.60	DV $0.80	DV $1.60
FF–FF	0.5		31	10	15	37	9	4	46	49	8.23	8.34
FF–FF	0.25		31	12	18	33	5	1	46	54	8.23	8.16
FF–FF	0		32	30	21	22	1	1	46	47	8.14	8.13
FF–FF	0.5	MEY	84	84	9	9	0	0	6	6	9.53	9.53
FF–FF	0.5	UFD	30	10	15	37	9	5	46	48	8.13	8.24
FF–FF	0.5	USA	32	25	14	28	8	5	47	43	8.25	8.44
FF–FF	0.5	$q \times 2$	33	11	6	27	12	17	49	45	7.70	8.55
FF–FT	0.5		41	20	12	31	2	2	44	47	8.18	8.31

(*b*) Case 2.

Simulation variations			ACE revenues (%)									
			Red cod (*Pseudophycis bachus*)		Elephantfish (*Callorhinchus milii*)		Deemed values on elephantfish (%)		Inframarginal profit (%)		Combined annual fishery rents (millions of dollars)	
TAC policy	Covariance	Other	DV $1.58	DV $3.16	DV $1.58	DV $3.16	DV $1.58	DV $3.16	DV $1.58	DV $3.16	DV $1.58	DV $3.16
FT–FF	0.5		38	38	4	4	0	0	58	58	3.53	3.53
FT–FF	0.25		39	39	3	3	0	0	57	57	3.45	3.45
FT–FF	0		41	41	3	3	0	0	57	57	3.37	3.37
FT–FF	0.5	$3 \times$ MSY	10	10	8	8	0	0	82	81	3.79	3.79
FT–FF	0.5	UFD	39	39	5	5	0	0	56	56	3.48	3.48
FT–FF	0.5	USA	37	37	6	6	1	1	56	56	3.55	3.55
FT–FF	0.5	$q \times 2$	31	28	23	34	6	2	40	36	3.80	3.84
FF–FF	0.5		7	7	8	9	1	1	84	84	3.80	3.80

Note: ACE, annual catch entitlement; SSB, spawning stock biomass; MSY, maximum sustainable yield; DV, deemed value charged per kilogram; FF, constant fishing mortality set at F_{MSY} except in MEY simulation; FT, fixed total allowable catch (TAC) set at deterministic MSY unless otherwise specified; MEY, maximum economic yield; UFD, uncertainty in fish distribution; USA, uncertainty in stock assessment; $q \times 2$, catchability coefficient for the bycatch species is doubled.

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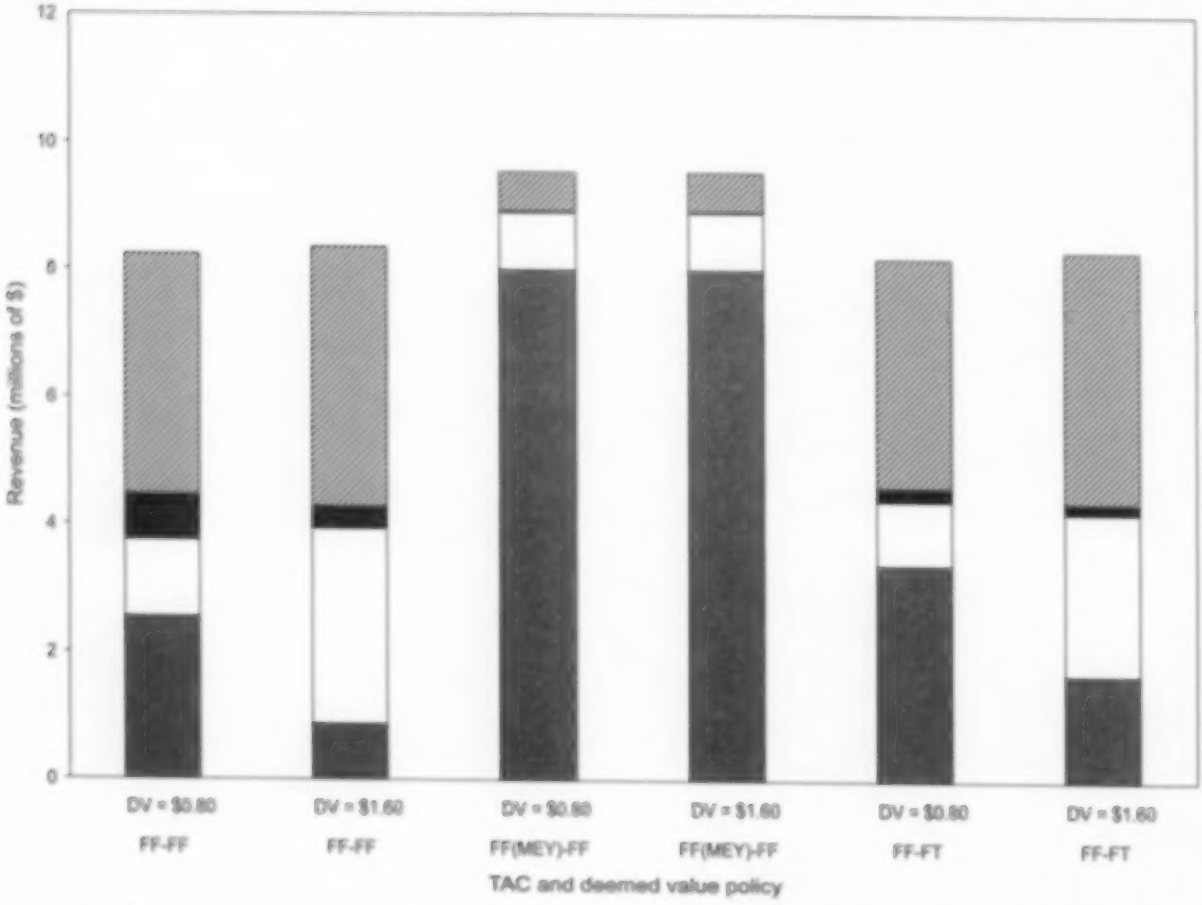
Fig. 5. Average orange roughy (*Hoplostethus atlanticus*) annual catch entitlement (ACE) revenues (shaded), smooth oreo (*Pseudocyttus maculatus*) ACE revenues (open), deemed value (DV) payments (solid), and inframarginal profits (hatched) for Case 1 with covariance of 0.5 under three total allowable catch (TAC) policies, each simulated with maximum DV on smooth oreo at \$0.80 and \$1.60. Columns 1–2 have a variable TAC with fishing mortality at the deterministic maximum sustainable yield (F_{MSY}) for both species. Columns 3–4 have a variable TAC for orange roughy with fishing mortality set at $0.65F_{MSY}$ and TAC for smooth oreo set at F_{MSY}. Columns 5–6 have a variable F_{MSY} TAC for orange roughy and a fixed MSY TAC for smooth oreo.



Reducing the target fishing mortality rate for orange roughy by 35% (to the MEY level, assuming high covariance) increases total fishery rents by over 15% to \$9.53 million despite a drop in average catches and revenues for both species (Table 3*a*, Fig. 5). These gains reflect lower total harvest costs. At the lower exploitation rate for orange roughy, it is no longer costly to stay within the smooth oreo TAC, and total DV payments fall to zero. Orange roughy ACE revenues increase dramatically to 84% of the total, while both inframarginal profits and smooth oreo ACE revenues decline substantially (in absolute terms as well as the share of total rents). Raising the maximum DV has no impact in this scenario.

Uncertainty in relative profitability across locations leads to a small overall decline in total fishery rents relative to the base case. This reflects the fact that it generally takes more effort and therefore higher harvest costs to take the orange roughy TAC. Neither smooth oreo ACE revenues nor total DV payments change substantially from the base case.

Relative to the base case, uncertainty in the stock assessment results in almost no change in total fishery rents when the maximum DV is \$0.80 and a slight increase in total fishery rents when the maximum DV is \$1.60. It does result in a decrease in the share of rents going to smooth oreo ACE owners, particularly with higher maximum DVs.

For the sensitivity analysis with the catchability coefficient on smooth oreo doubled, total fishery rents decline about 7% relative to the base case when the maximum DV is \$0.80. This reflects a reduction in average catches and revenues from smooth oreo due to overfishing. However, with a maximum DV of \$1.60, total fishery rents are increased slightly relative to the base case. The greater difficulty of avoiding smooth oreo bycatch and the penalty of high DVs serve to reduce total orange roughy catch and average effort and move the orange roughy fishery closer to MEY. Although the share of rents accruing to orange roughy ACE owners is little different from the base case, there is a shift of rents away from smooth oreo ACE revenues to DV payments rela-

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tive to the base case. Also, unlike other scenarios, DV payments increase as the maximum DV is increased.

The scenario with a fixed TAC for smooth oreo set equal to deterministic MSY results in slightly lower total fishery rents than the base case model where both TACs are maintained at F_{MSY}. However, this TAC-setting strategy generally increases orange roughy ACE revenues while reducing DV payments and smooth oreo ACE revenues.

Case 2: biological risk and stock productivity

No major biological risk to the elephantfish stock is evident in any of the scenarios modeled for this case. Neither the SSB of red cod nor that of elephantfish ever falls below 10% of B_0 or 50% R_{max}, and both stay above 20% of B_0 more than 95% of the time. These results hold for several sensitivity analyses where (*i*) the TAC for red cod is set at three times the deterministic MSY; (*ii*) there is uncertainty in relative profitability across locations or in stock assessments; and (*iii*) the catchability coefficient on elephantfish is doubled.

Catches of elephantfish generally remain within the TAC even with the lower DV mainly because catches of red cod are well below the TAC in most years. These simulated results are consistent with reality. The simulations were calibrated by adjusting harvest costs and catchability to achieve an average catch and exploitation rate consistent with observed values in the fishery. In most years, the fishery is constrained by economics rather than by regulations. Fishing is constrained to locations with high catch rates because it is unprofitable to fish locations where catch rates are low.

Case 2: economic efficiency and distribution of resource rents

For the base case scenario, with a covariance of 0.5 and the red cod TAC fixed at MSY while the elephantfish TAC varies to maintain F_{MSY}, average annual total fishery rents are $3.53 million. Total rents and their distribution are unchanged by doubling the maximum DV on elephantfish from $1.58 to $3.16. Although elephantfish accounts for about 20% of average gross revenues, elephantfish ACE revenues are only 4% of the total. Red cod ACE revenues are 38% of the total, and inframarginal profits account for 58% (Table 3*b*, Fig. 6). No DV is paid. Results are changed only slightly with a lower covariance of stock distributions (e.g., 0.25 or 0). Total fishery rents are slightly lower, and the share of rents accruing to red cod ACE owners is a percentage point higher.

Unlike the previous case, the fixed red cod TAC that yields the highest total annual fishery rents is above rather than below the deterministic MSY. In fact, total fishery rents continue to increase up to the point where the TAC for red cod is increased by a factor of three. The higher TAC removes constraints on the catch of red cod when its biomass is very high and it is profitable to catch. Harvests costs are sufficient to ensure the stock is never overexploited. Total fishery rents with a fixed TAC for red cod set at three times the deterministic MSY and an F_{MSY} policy for elephantfish are $3.8 million, roughly a 7% increase from the base case. The distribution of rents is substantially different from the base case. Red cod ACE revenues decrease from 38% to 10% of total rents, while elephantfish ACE revenues increase from 4% to 8%. Inframarginal profits increase to 82%.

Adding uncertainty in relative profitability across locations results in a small decrease in total rents as a result of higher harvest costs. The reduction is in inframarginal profits; ACE revenues for both species are fairly stable. For the base case, stock assessment uncertainty for elephantfish actually increases total fishery rents and shifts some rents away from red cod ACE revenues into DV payments and elephantfish ACE revenues.

If the catchabilty of elephantfish is doubled, total fishery rents are 7% to 9% higher than in the base case with maximum DVs of $1.58 and $3.16, respectively. A much greater share of rents (23% and 34%, respectively) are attributable to elephantfish ACE revenues and DVs at the expense of red cod ACE revenues and inframarginal profits.

Although adjusting the TAC of red cod to maintain it at F_{MSY} is unrealistic, we consider this scenario for comparative purposes. The results are similar to the scenario with the red cod TAC fixed at three times MSY. Total fishery rents increase to $3.8 million, and rents shift from red cod ACE revenues to inframarginal profits and elephantfish ACE revenues.

Discussion

The DV system provides an economic incentive to keep catches of bycatch stocks within TACs, but allows those TACs to be exceeded when the cost of reducing bycatch is particularly, or unexpectedly, high. This flexibility can increase the risk of overexploiting a bycatch stock and threatening its viability. A key determinant of this risk, as illustrated in both cases we examine, is whether the target exploitation rate of the bycatch is lower than that of the target stock. When the exploitation rate of the target species is higher than that of the bycatch species, and the distribution of the two species is highly correlated, it is possible to overexploit the bycatch species even without targeting it. Raising the DV to the ex-vessel price of the bycatch species in response to overcatch theoretically eliminates incentives to target the bycatch species, but does not create incentives to actively avoid it. The bycatch species may be overexploited simply as a byproduct of harvesting the target species. The risk of overexploitation is aggravated if the fish become vulnerable to the fishery before they mature, as in the case of smooth oreo (our first case). If, however, there is a low spatial correlation between the target and bycatch species, eliminating incentives to target the bycatch should be sufficient to avoid overexploitation.

For the cases modeled, allowing DVs to rise to twice the ex-vessel price in response to TAC violations eliminates the risk of overexploitation, as long as both TACs are adjusted annually to maintain fishing mortality at or below F_{MSY}. Higher DVs provide sufficient incentives for fishers to actively avoid bycatch and generally keep catches within the TACs, with only temporary exceptions. This is true even when the distribution of stocks is highly correlated, so that bycatch avoidance is costly. An exception to this may be if the catchabilty of the bycatch stock is higher than that of the target stock (i.e., in terms of the percentage of the fish biomass in a given area taken with a unit of fishing effort). The

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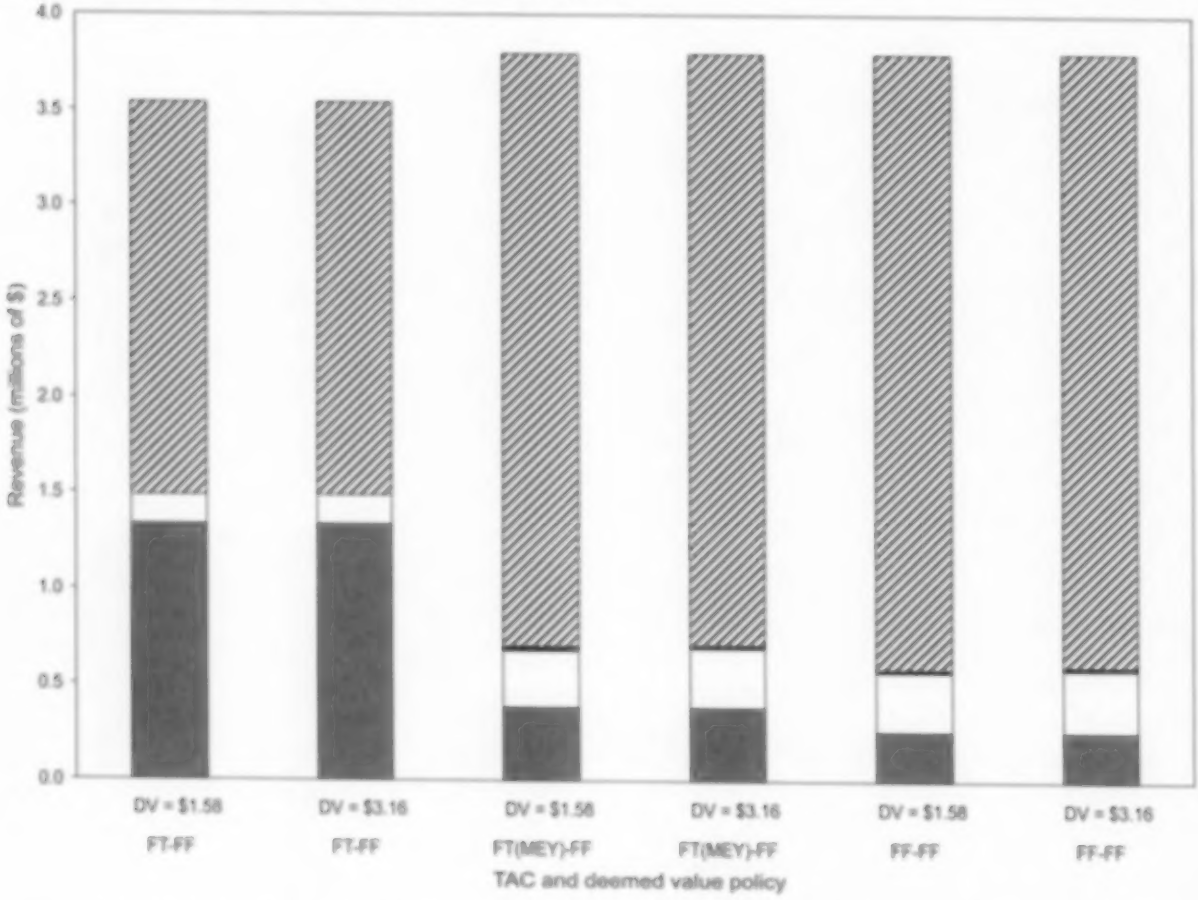
Fig. 6. Average red cod (*Pseudophycis bachus*) annual catch entitlement (ACE) revenues (shaded), elephantfish (*Callorhinchus milii*) ACE revenues (open), deemed value (DV) payments (solid), and inframarginal profits (hatched) for Case 2 with covariance of 0.5 under three total allowable catch (TAC) policies, each simulated with maximum DV on elephantfish at $1.58 and $3.16. Columns 1–2 have a fixed TAC for red cod set at maximum sustainable yield (MSY) and a variable TAC for elephantfish with fishing mortality set at the deterministic MSY (F_{MSY}). Columns 3–4 have a fixed TAC for red cod set at three times MSY and a variable F_{MSY} TAC for elephantfish. Columns 5–6 have a variable F_{MSY} TAC for both species.



reverse relationship is actually more likely, since harvest gear has presumably been designed specifically to maximize catch of the target species. In that case, the risk of bycatch overexploitation would be reduced relative to our simulations.

Ideally, DVs can be maintained below ex-vessel price so that they discourage intentional catches above the TAC, but maintain the incentive to land (rather than discard) unintended catch. For most stocks, DVs have been kept below ex-vessel price for this reason. However, our simulations demonstrate it is sometimes necessary to raise DVs well above ex-vessel prices to constrain bycatch. This can create strong incentives for illegal discarding. If not deterred, discarding could reduce fishery rents and increase risk of overexploitation. Furthermore, data quality may degenerate, making stock assessments less precise. When high DVs create incentives for illegal discarding, it may be advisable to use onboard observers or other mechanisms to increase compliance with discarding prohibitions.

Fixed TACs for the bycatch stock generally increase biological risk, particularly if the bycatch species is vulnerable to fishing before maturity or if it is less productive than the target stock. Higher maximum DVs reduce but do not eliminate this risk because depletion can occur even when catches are kept with the TAC. For the bycatch stock, total catch is likely to decline as stock declines, but under a fixed TAC there may actually be a decreasing incentive to avoid it. As biomass falls, it becomes easier to keep catch within the TAC, and the ACE price for the bycatch species will fall, reducing incentives to avoid it. Although it did not occur with any of the cases we modeled, the bycatch stock in theory could be driven to extinction if the TAC is not reduced when the stock declines.

Neither of our cases suggests that increasing DVs to well above ex-vessel price in response to TACs violations reduces overall fishery rents. This is true even for cases where abundance of the target or bycatch stocks is highly variable. The

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DV policy has surprisingly little impact on combined fishery rents even when DV rates on the bycatch species are increased to twice the ex-vessel price. In some cases, combined fishery rents actually increase with higher DVs because fishing intensity is reduced, bringing the fishery closer to MEY.

A more conservative harvest strategy for the target species, such as the MEY policy in Case 1, can increase total fishery rents as well as the share of rents accruing to ACE owners of the target species. A lower exploitation rate allows biomass of the target stock to grow, reducing the nominal effort required to take the TAC. Even if effort were randomly distributed vis-à-vis the bycatch stock, this would be expected to reduce total bycatch unless the bycatch stock had grown proportionately. The reduction in bycatch and potentially other environmental impacts of fishing, not to mention lower risk of overexploiting the target stock, provide an additional impetus to reduce exploitation rates below MSY levels. The simulations also suggest that quota owners of the target stock may substantially increase their share of fishery rents by supporting a more conservative harvest strategy. As Case 1 shows, an MEY policy not only increases overall fishery rents, but also results in a substantial shift away from inframarginal profits and bycatch ACE revenues and into ACE revenues for the target species.

If a stricter DV policy is imposed on a fishery where bycatch regularly exceeds the TAC, total fishery rents are likely to decline initially and rebound only as the target stock's biomass increases and harvest costs fall. Our simulations did not look at the present value of imposing a stricter DV policy on a fishery that had been operating under a more lenient policy. For a slow-growing target species, the benefits of a lower exploitation rate may not be realized for some time, and the present value of the policy change could be negative despite an increase in long-run average rents. Higher maximum DVs can also increase the variability of catches and rents over time. This is generally undesirable though the economic impact is hard to quantify.

The distribution of rents can be quite sensitive to the TAC-setting policy and DV policy, even if total rents are not. This may be less important if all fishers have balanced quota portfolios, but generally there is heterogeneity in quota portfolios, so there will be winners and losers. Stricter DV policies (i.e., with higher maximums) generally shift rents from owners of target species quota to owners of bycatch species quota.

New Zealand has recently introduced a number of species taken primarily as bycatch and is expected to introduce more in the future. In the past, bycatch of these species was essentially unregulated. Once they are in the quota system, fishers must balance their landings with ACE. Strict DV policies that create strong penalties for exceeding TACs can be expected to shift value out of quota for existing target species into quotas of these new species, even if they have little commercial value, simply because they constrain the ability to utilize target species quota. Since much of the quota for these new quota species is being auctioned rather than given to existing quota owners based on recent catch histories, the introduction of these new species to the quota management system may shift value from existing quota assets to the government.

With few exceptions, the simulations showed that higher DVs rates actually reduce the total DV payments. Quota violations are elastic with respect to the DV price; the magnitude of the TAC violation decreases by a larger percentage than the DV rate increases. This might not hold if harvesters cannot easily control bycatch rates, but even then they might choose to reduce target catch to avoid punitive DVs. DV revenues currently accrue to the government in New Zealand. This creates a moral hazard problem for the government, which can increase its DV revenues by lowering the DV rate. By doing so, they effectively undercut the ACE price that would prevail if catch were strictly limited to the TAC.

As a benchmark, we assumed perfect information on the part of harvesters about the catches of both target and bycatch species across all locations; we also assumed that harvesters would correctly choose the most profitable options. Our simulations suggest that greater uncertainty in relative profitability of locations that could be caused by uncertainty in catch rates may increase the risks of breaching reference points. Uncertainty in relative catch rates across locations also reduces total fishery rents slightly, as one would expect, but this impact is small and the distribution of rents is virtually unchanged. In some cases, the actual uncertainty in catch rates, and consequently relative profitability, might be considerably higher than the levels we include in our sensitivity analysis. If uncertainty were sufficiently high, it might become necessary to reduce bycatch by forgoing use of target quota. Ironically, this might increase fishery profits if the target fishery TAC is higher than MEY. The impacts of uncertainty in stock assessments on biological risk and efficiency are ambiguous and provide little insight into how this factor might influence either the risk or efficiency associated with various DV policies.

Our model implicitly assumes competitive markets cause bycatch ACE prices to converge on the marginal value of an increase in the TAC. This causes all fishers to consider the true opportunity costs of bycatch ACE when choosing between fishing alternatives and leads catches of both species that maximize total fishery rents in a time period, given the TAC and DV constraints. In reality, the initial ACE price at the beginning of the season might be too high or too low but would adjust toward the market-clearing level as the year went on. Too high an initial price would leave surplus quota, causing downward pressure on prices; a price set too low would provide insufficient incentives to reduce bycatch and would lead to catch rates that threaten to bust the TAC. This would create upward pressure on the ACE price by those who would otherwise have to pay DVs (as long as the ACE price does not exceed the DV). As long as ACE prices are not systematically biased up or down from those arising under perfect information and competitive markets, the effect of this evolution of ACE prices during a season should be somewhat similar to the effect of uncertainty in relative catch rates; it effectively changes the relative profitability of fishing alternatives in the same way. This uncertainty tends to decrease fishery rents and may increase the risks of overexploiting the bycatch stock.

In summary, we find that allowing TACs to be exceeded through a DV system will generally not create substantial risks as long DVs are increased in response to TACs being

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exceeded and TACs are reduced in response to stock declines. However, it may be necessary to raise DVs to well above ex-vessel prices in some cases, which is likely to necessitate a strong compliance program to deter illegal discarding. Allowing DVs to rise to well above ex-vessel prices is unlikely to reduced overall efficiency but will cause rents to shift from owners of target species quota to owners of bycatch species quota. This can be avoided by setting more conservative TACs for the target species.

These simulations modeled only cases with one target species and one bycatch species. Some of the conclusions drawn from this analysis may not apply to more complex multispecies fisheries. For example, if there are multiple bycatch species that vary independently, this may increase the costliness of keeping bycatch below TACs, since avoiding bycatch of one species could increase bycatch of another. Modeling DV policies for fisheries with more than two species would be a useful extension of this analysis.

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Appendix A. Biological model.

The number of individuals N of stock i and age j in year t is

$$(A1)\quad N_{i,j,t+1} = \begin{cases} N_{i,0,t+1} & \text{if } j = 0 \\ N_{i,j-1,t}\exp(-Z_{i,j-1,t}) & \text{if } 1 \le j \le J \\ N_{i,J-1,t}\exp(-Z_{i,J-1,t}) & \\ \quad + N_{i,J,t}\exp(-Z_{i,J,t}) & \text{if } j = J \end{cases}$$

where $Z_{i,j,t}$ is the instantaneous total mortality rate; it is the sum of natural mortality M_i and fishing mortality $F_{i,j}$. The Jth cohort is a plus group where the number in the cohort is adjusted according to total mortality for the year and the survivors from cohort $j - 1$ are added. The number of recruits $N_{i,0}$ is determined by a stochastic Beverton and Holt recruitment function:

$$(A2)\quad N_{i,0,t+1} = \frac{B^S_{i,t}}{(\alpha + \beta B^S_{i,t})}\exp(\varepsilon'_{i,t} - \sigma'^2_{i,t}/2),$$

$$\varepsilon'_{i,t} \sim N(0, \sigma'^2_{i,t})$$

where $B^S_{i,t}$ is the spawning stock biomass (SSB) for species i, and ε is a random number with zero mean and unit variance and σ'_i standard deviation of recruitment for species i. The

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parameters α and β are themselves parameterized by the steepness of the stock–recruitment function (h), average SSB with no fishing (B_0), and the corresponding average recruitment (R_0), which is calculated by dividing B_0 by SSB per recruit with zero fishing mortality according to

$$\text{(A3)} \quad \alpha = B_0(1-h)/(4hR_0), \qquad \beta = (5h-1)/(4hR_0)$$

SSB is calculated by summing the weight of mature fish in all cohorts given the maturity ogive $A_{i,j}$, which is determined by

$$\text{(A4)} \quad A_{i,j} = \begin{cases} 2^{-[(j-A_i^1)/(A_i^1-A_i^L)]^2} & \text{if } j < A_i^1 \\ 1 & \text{if } j \geq A_i^1 \end{cases}$$

where the proportion mature is 100% at A_i^1, maturity is 50% at $j = A_i^1 - A_i^L$.

The model first calculates a total weight of catch and then calculates the total number of fish caught from each cohort, $Y_{i,j}$, by multiplying the number of recruited fish in each cohort by the ratio of total harvest weight to the total recruited biomass:

$$\text{(A5)} \quad Y_{i,j,t}^R = H_{i,t} \frac{S_{i,j}N_{i,j,t}}{\sum_{k=0}^{J} w_{i,k}S_{i,k}N_{i,k,t}}$$

where individual fish weights $w_{i,j}$ are calculated from standard von Bertalanffy length-at-age equations and length–weight conversions (Table 1), and the recruited proportion of each cohort is determined by the selectivity ogive $S_{i,j}$:

$$\text{(A6)} \quad S_{i,j} = \begin{cases} 2^{-[(j-S_i^1)/(S_i^1-S_i^L)]^2} & \text{if } j < S_i^1 \\ 1 & \text{if } j \geq S_i^1 \end{cases}$$

where the parameters S_i^1 and S_i^L are analogous to A_i^1 and A_i^L for the maturity ogive.

Survival from each cohort is dependent on the total mortality rate $Z_{i,j}$, which is the sum of (exogenous) natural mortality and (endogenous) fishing mortality. The cohort-specific fishing mortality $F_{i,j}$ is estimated using a simple algorithm that calculates fishing and total mortality consistent with the known population and catch numbers-at-age, the natural mortality rate, and the standard Baranov catch equation:

$$\text{(A7)} \quad Y_{i,j,t} = N_{i,j,t} \frac{F_{i,j,t}^*}{Z_{i,j,t}} [1 - \exp(-Z_{i,j,t})]$$

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Characterization of phosphorus in sequential extracts from lake sediments using ^{31}P nuclear magnetic resonance spectroscopy

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Abstract: Phosphorus (P) compounds in three different lake surface sediments were extracted by sequential P extraction and identified by ^{31}P nuclear magnetic resonance (^{31}P NMR) spectroscopy. The extraction procedure primarily discriminates between inorganic P-binding sites but most extraction steps also contained P not reacting (nrP) with the molybdenum complex during P analyses. In all three lakes, the nrP dominated in the NaOH extracts. Nonreactive P from the dystrophic lake was dominated by potentially recalcitrant P groups such as orthophosphate monoesters, while the nrP in the two more productive lakes also contained polyphosphates, pyrophosphate, and organic P groups such as P lipids and DNA-P that may be important in remineralization and recycling to the water column. In addition, polyphosphates showed substantial dynamics in settling seston. The Humic-P pools (P associated with humic acids) showed strong signals of orthophosphate monoesters in all three lakes, which supported the assumption that P-containing humic compounds are indeed recovered in this fraction, although other organic P forms are also present. Thus, in addition to expanding the understanding of which organic P forms that are present in lake sediments, the ^{31}P NMR technique also demonstrated that the chemical extraction procedure may provide some quantification of recalcitrant versus labile organic P forms.

Résumé : Nous avons extrait les composés phosphorés (P) des sédiments lacustres superficiels de trois lacs différents par extraction séquentielle du P et les avons identifiés par spectroscopie de résonnance magnétique nucléaire ^{31}P (^{31}P NMR). Le processus d'extraction discrimine surtout entre les divers sites de liaison du P inorganique, mais la plupart des étapes de l'extraction contiennent aussi du P (nrP) qui ne réagit pas au complexe de molybdène durant l'analyse de P. Dans les trois lacs, le nrP domine dans les extraits au NaOH. Le P non réactif dans le lac dystrophe est dominé par les groupes P potentiellement récalcitrants, tels que les monoesters d'orthophosphate, alors que le nrP dans les deux lacs plus productifs contient aussi des polyphosphates, du pyrophosphate et des groupes organiques de P, tels que les P-lipides et l'ADN-P qui peuvent être d'importance pour la reminéralisation et le recyclage vers la colonne d'eau. Il y en plus une importante dynamique des polyphosphates dans le seston en sédimentation. On trouve dans les pools de P humique un fort signal de monoesters d'orthophosphate dans les trois lacs, ce qui appuie l'hypothèse selon laquelle les composés humiques qui contiennent du P sont en fait récupérés dans cette fraction, bien que d'autres formes organiques de P soient aussi présentes. Ainsi, non seulement la technique ^{31}P NMR permet de mieux connaître les formes organiques de P présentes dans les sédiments lacustres, mais elle démontre que la procédure d'extraction chimique peut fournir un bilan quantitatif approximatif des formes organiques de P récalcitrantes par rapport aux formes labiles.

[Traduit par la Rédaction]

Introduction

As phosphorus (P) is a key nutrient in regulating primary production in most lakes, numerous studies have focused on the turnover of different P forms in lake sediments (e.g., Golterman 2004 and references therein). Although organic P may constitute a major proportion of sediment P (Turner et al. 2005) that originates from both external sources and autochthonous production, most studies have focused on inorganic P forms (e.g., Golterman 1996; Rydin 2000; Reitzel et al. 2003). One reason for this unbalanced attention is the lack of proper techniques for extraction and identification of

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Table 1. Water and sediment chemistry of the three lakes.

Parameter	Lake Sønderby	Lake Erken	Lake Kvie
Water			
Trophic status	Eutrophic	Mesotrophic	Dystrophic
TP (μmol·L^{-1})	4.2	0.71	2.8
Alkalinity (mequiv.·L^{-1})	2.74	1.79	0.04
Sediment			
LOI (%)	45	20	24
Inorganic P (%)	64	68	40
Organic P (%)	36	32	60

Note: TP, total phosphorus; LOI, loss on ignition.

organic P forms in sediments. As a result, organic P has mainly been described in relation to various sequential extraction procedures (e.g., Hieltjes and Lijklema 1980; Psenner and Pucsko 1988; Paludan and Jensen 1995), primarily developed to quantify inorganic sedimentary P forms.

Two decades ago, Newman and Tate (1980) introduced the ^{31}P nuclear magnetic resonance (NMR) spectroscopy to the field of ecology by extracting soil samples in alkaline solutions and subsequently using ^{31}P NMR spectroscopy to identify P compounds. ^{31}P NMR spectroscopy distinguishes different P groups based on specific resonance frequencies, reflecting the chemical environment surrounding the P nuclei. The method can seldom be used to identify single P compounds, but is very efficient at defining different P groups based on their binding properties. Thus, it is possible to differentiate between P bound as, e.g., orthophosphate, orthophosphate monoesters, orthophosphate diesters, pyrophosphate, polyphosphates, and phosphonates.

In this study, we explore the forms of P extracted by traditional, sequential P fractionation by analysing the extracts using ^{31}P NMR spectroscopy on surface sediment from three lakes of different trophic state. Our aim is to increase the resolution beyond dividing extracted P into "reactive" or "nonreactive", referring to P detected by the molybdenum blue method before and after digestion of the extract, and subsequently to be able to better understand which part of the P not reacting (nrP) that is more recalcitrant and which part is less. Such understanding may especially be relevant for oligotrophic lakes in forested watersheds, since extractable nrP in such lakes sediments may contribute more than 60% of the total P (Jensen et al. 2005).

Materials and methods

Study sites

Lake Sønderby is a small (8 ha, mean depth = 2.8 m, maximum depth = 5.7 m), eutrophic, polymictic, hardwater lake (Table 1) situated in the southern central part of Denmark (55°11′N, 9°21′E). The catchment area is 122 ha, with farmland contributing 85%, wetland 6%, and urban 9%. Inlets are through subsurface drainpipes, and outlet occurs through seepage (90%) and by pumping at high water levels in winter (10%). In 2001, the lake was treated with $AlCl_3$ to reduce the internal P loading. The lake experiences periods of anoxia during the summer.

Lake Kvie (55°44′N, 8°45′E) is a low pH (5.5), dystrophic, softwater lake (30 ha, mean depth = 2 m, maximum depth = 2.6 m) situated in the southern part of Denmark (Table 1). The catchment area is 57 ha, with farmland contributing 35%, wetland 51%, and forest (14%). The lake is one of the few lakes in Denmark housing a population of isoetids. Oxygen is always present at the sediment surface.

The mesotrophic Lake Erken (59°51′N, 18°35′E) is located in the southeastern part of Sweden, and has a surface area of 2400 ha, a maximum depth = 21 m, with an average depth = 9 m (Table 1). The lake has been mesotrophic (total P (TP) ~27 μg·L^{-1}) since measurements started in 1930, as the lake is situated on nutrient-rich glacial and postglacial clay deposits. It is assumed that the external P load to Lake Erken has remained fairly constant for at least two centuries (Rydin 2000), because of the small and constant number of inhabitants in the mostly (~70%) forested catchment area that covers 137 km^2, of which 10% is low-intensity farmland areas. The lake stratifies during the summer, resulting in periods of bottom water anoxia.

Eight surface sediment cores (0–1 cm) were collected from the deepest part of each lake with a Kajak corer in April 2004. Sediment cores from each lake were pooled before analysis to obtain representative samples from the sampling areas and to obtain enough sediment for the ^{31}P NMR analysis. Sediment was collected from areas in the lakes that had been sampled before, and surface sediment TP measured in this study differed only little from other measurements from the same years (Lake Erken: standard error of the mean (SEM) < 5%; Lake Sønderby: SEM < 1%; Lake Kvie: SEM < 4%; K. Reitzel and H.S. Jensen, unpublished data). Thus, we assume that the low variation in TP and the high number of pooled sediment subsamples make the composed samples highly representative for each lake and thereby justify a tentative comparison of the P groups among them, even though no statistics can be made.

In September 2004, four sediment traps (Ø = 30 cm, height = 60 cm, no poison) were deployed in Lake Erken to collect settling material at 8 m depth and 15 m depth. The traps were deployed at the same location as the sediment sampling and were emptied after 30 days. Settling seston was collected with a 40 μm plankton net in the upper 1 m of the lake on the same day the sediment traps were deployed.

P fractionation

A sequential extraction scheme proposed by Psenner and Pucsko (1988) was followed in principle with an additional acidification (pH = 1) of the NaOH extract with 2 mol·L^{-1}

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H_2SO_4 to precipitate P associated with humic acids (Humic-P) (Paludan and Jensen 1995). The extraction procedure yielded six operationally defined P pools in the sediment (Table 2). Thus, porewater P (P_{Water}) was extracted in demineralized water, after which iron-bound P (Fe-P) was extracted in bicarbonate-buffered dithionite (P_{BD}). In step 3, a major part of the biogenic P such as organic P (Ahlgren et al. 2005) and polyphosphates (Hupfer et al. 2004) was extracted along with reactive P bound to aluminium hydroxides (Al-P) (e.g., Reitzel 2005). Phosphorus, assumed to be associated with humic acids, was precipitated by adding 2 mol·L^{-1} H_2SO_4 to the NaOH extract (Paludan and Jensen 1995). The precipitate was collected following centrifugation of the acidified NaOH extract and subsequently redissolved in 0.1 mol·L^{-1} NaOH, after which Humic-P was determined by inductively coupled plasma (ICP) spectroscopy. The NaOH-extractable P remaining in solution is termed P_{NaOH} and contains Al-P and dissolved organic P such as, e.g., fulvic acids (Paludan and Jensen 1995). In step 4, acid-soluble P, mainly bound to Ca (P_{HCl}), was extracted. The residual P (Res-P) was calculated as the difference between the TP in the sediment and the sum of the extracted P pools.

TP and molybdenum-reactive P (rP) were measured in all extracts. TP was measured as rP in the extracts after wet oxidation with $K_2S_2O_8$ for 1 h at 120 °C (Koroleff 1983). rP was measured by colorimetric analysis, according to Koroleff (1983). The difference in TP and rP in each extract is termed nrP, and denotes P not reacting with the molybdenum complex. All P groups except orthophosphate were considered as nrP, when the nrP was estimated from the ^{31}P NMR spectra. Sediment dry weight was measured by freeze-drying the sediment to constant weight. Loss on ignition was measured by combusting the freeze-dried sediment at 550 °C for 6 hours.

Total sediment P was measured by autoclaving combusted sediment in 1 mol·L^{-1} HCl at 120 °C for 1 h followed by rP measurement for determination of P. Total amounts of organic P in the sediments were calculated as the sum of the nrP, Humic-P, and Res-P after subtracting inorganic polyphosphates and pyrophosphates from the nrP.

Preconcentration

As the P concentrations in the sediment extracts were generally too low for ^{31}P NMR analysis, preconcentration was required. The commonly used preconcentration techniques are rotary evaporation (e.g., Hupfer et al. 2004) or freeze-drying of the extract followed by redissolution in NaOH–EDTA (e.g., Cade-Menun et al. 2002). The possible effects of these preconcentration techniques on P groups extracted and recovery of P in the concentrated extracts were tested prior to ^{31}P NMR analysis of the lake sediments. To compare with literature results, we tested the preconcentration techniques on NaOH–EDTA extracts. Thus, surface sediment from Lake Erken was extracted in NaOH–EDTA, and from this extract, two groups of three replicates were either preconcentrated 10 times by rotary evaporation or by freeze-drying and subsequently centrifuged at 15 000 r·m^{-1} (1 r = 2π rad; 23 646*g*) for 10 min, after which they were analyzed by ^{31}P NMR analysis and ICP to evaluate whether rotary evaporation would be less destructive and give a higher re-

Table 2. Extraction procedure.

Operational P pools	pH	Extractant	Main extraction time (h)	Repetitions[a]	P compounds extracted
P_{Water}	~7	H_2O	1	1	Pore water P + nrP
P_{BD}	~7	0.11 mol·L^{-1} $NaHCO_3$ + 0.11 mol·L^{-1} $Na_2S_2O_4$	1	2	Fe-P + nrP
P_{NaOH}	~12	0.1 mol·L^{-1} NaOH	16	1	Al-P + nrP
Humic-P	~1	0.1 mol·L^{-1} NaOH + 2 mol·L^{-1} H_2SO_4	48[c]	—	P associated with humic acids
P_{HCl}	~1	0.5 mol·L^{-1} HCl	1	1	Inorganic Ca-P
Res-P	—	Calculated[b]	—	—	P not extracted in previous steps

Note: 1 g of wet sediment was extracted sequentially in 25 mL of each extractant. The sediment pellet was washed in 25 mL of deionized water before each new extractant was used. This wash was used to avoid readsorption of P onto nonextracted phases and was included in the previously extracted P pool. After each repetition, a 5 min centrifugation was performed. BD, bicarbonate-buffered dithionite; nrP, phosphorus not reacting with the molybdenum complex.

[a]Res-P is calculated as the difference between total phosphorus (TP) and the sum of the operational P groups.

[b]Each repetition after the main extraction time was 5 min.

[c]Time of precipitation.

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covery of P groups compared with freeze-drying. According to a Kolmogorov–Smirnoff test performed in SigmaStat 2.3 (SPSS, Chicago, Illinois), the data were normal distributed, and replicate observations of three could then be compared by parametric *t* tests in SigmaStat 2.3 to determine whether differences in P recovery and in extracted P groups between freeze-dried and rotary-evaporated samples were significant (critical p value = 0.05). In cases where the concentration of the extracted P groups were below the detection limit, a concentration of 0 $\mu mol \cdot g^{-1}$ dry weight was assumed. In these cases, data were tested by a one sample *t* test. Estimates of variation are given as SEMs.

^{31}P NMR analysis

Extracts containing nrP (Table 3) were analyzed by ^{31}P NMR spectroscopy. The sediment (~100 g) was extracted according to the sequential sediment extraction procedure, with a few modifications. To achieve P concentrations high enough for ^{31}P NMR analysis, sediment to extractant ratios were increased from 1:25 to 1:3, and the water extraction was omitted, as this pool was negligible in all three lakes (Table 3). Only one BD extraction was conducted, as the P concentrations in the BD extracts would otherwise be too low for ^{31}P NMR analysis. Furthermore, pH in the oxygenated BD extracts had to be raised to ~13, to improve the resolution, as broadening of peaks (line broadening) otherwise caused spectra of poor quality.

Acidification of the NaOH extract for precipitating Humic-P resulted in poor spectral resolution, probably caused by high salt concentration (Baldwin 1996). Therefore, ^{31}P NMR analysis on the NaOH extracts prior to the acidic precipitation of humic acids was performed. The humic precipitate was analysed by ^{31}P NMR after redissolving it in 0.1 $mol \cdot L^{-1}$ NaOH.

Material from the sediment traps and the settling seston was prepared the same day and sampled in a way similar to the sediment samples extracted in NaOH.

Subsamples of the extracts for ^{31}P NMR were collected for measurement of TP by ICP prior to a 10 times preconcentration of the extracts by rotary evaporation at 45 °C. For the ^{31}P NMR analysis, the samples were centrifuged at 15 000 $r \cdot m^{-1}$ (23 646*g*) for 10 minutes to remove particles that would potentially cause line broadening of the spectra. Then 5% (*v*/*v*) of the BD solution was added to the samples prior to measurement to reduce interference from paramagnetic ions such as oxidized species of Fe and Mn (Cade-Menun et al. 2002; Ahlgren et al. 2005). Prior to measurements, 20% (*v*/*v*) of D_2O was added to the samples to obtain a stable lock signal.

The ^{31}P NMR spectra were measured at 121.5 MHz on a 300 MHz Varian MercuryPlus NMR spectrometer at ambient temperature. Spectra were recorded using a 63° observe pulse, acquisition time 0.4 s, relaxation delay 1.2 s, acquiring around 30 000 transients (12 h). These settings were tested and found adequate by Ahlgren et al. (2005). Chemical shifts were indirectly referenced to external 85% H_3PO_4 (at $\delta = 0.0$) via the lock signal. To obtain peak areas, peaks in the raw spectrum, with a signal to noise ratio exceeding 4, were fitted with Lorentzian line shapes using the deconvolution subroutine of the NMR software (Vnmr 6.1C, Palo Alto, California). Spectra were plotted with a line broaden-

Table 3. P pools based on sequential extraction of surface sediment in the three lakes.

	Lake Sonderby						Lake Erken						Lake Kvie					
	rP		nrP		Sum		rP		nrP		Sum		rP		nrP		Sum	
P pool	Conc.	%	Conc.	%	Conc.	%	Conc.	%	Conc.	%	Conc.	%	Conc.	%	Conc.	%	Conc.	%
P_{Water}	3	2	2	1	5	3	1	1	0	0	1	1	1	1	0	0	1	1
P_{BD}	27	15	3	2	30	17	21	26	0	0	21	26	6	8	2	3	8	11
P_{NaOH}	55	31	18	10	73	41	15	18	7	9	22	27	21	28	6	8	27	36
Humic-P	—	—	7	4	7	4	—		6	7	6	7	—	—	10	13	10	14
P_{HCl}	17	10	0	0	17	10	9	11	0	0	9	11	1	1	0	0	1	1
Res-P	45	25	—	—	45	25	23	28	—	—	23	28	28	37	—	—	28	37
TP					178						82						75	

Note: Concentrations (Conc.) are in $\mu mol \cdot g^{-1}$ dry weight. The contributions of each P pool to the total amount of P in the sediment are given in percentages. *rP*, molybdenum-reactive phosphorus; *nrP*, phosphorus not reacting with the molybdenum complex; BD, bicarbonate-buffered dithionite; Res-P, residual phosphorus; TP, total phosphorus.

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ing of 10 Hz. From these peak areas, the contribution of the individual P groups was calculated relative to the TP in the extracts. Assignment of peaks was done using standard solutions of orthophosphate ($Na_2HPO_4 \cdot 7H_2O$) and pyrophosphate ($Na_2P_2O_7 \cdot 10H_2O$) added to the sediment extracts, as well as by comparisons with literature.

Results

P fractionation

P fractionation showed that P_{Water} was low in all sediments, and nrP in this fraction was only found in the extracts from Lake Sønderby (Table 3). The P_{BD} made up between 11% and 26% of the TP in the sediments, and the extracts of Lake Sønderby and Lake Kvie both contained some nrP, whereas the P_{BD} in Lake Erken was composed of rP solely. The P_{NaOH} was the dominant P pool in all three lakes and also the P pool containing most nrP. Humic-P constituted between 4% and 14% of the TP in the sediment, with the highest amount extracted in Lake Kvie. In Lake Kvie, the P_{HCl} made up 1% of the TP, whereas P_{HCl} made up around 10% in Lake Sønderby and Lake Erken. P_{HCl} only extracted rP. The Res-P varied between 25% and 37% of the TP, with the highest amount found in Lake Kvie sediment.

Preconcentration

Rotary evaporation resulted in a significantly higher recovery of TP in the concentrated extracts (65%) compared with freeze-drying (50%) (Fig. 1). Furthermore, middle groups of polyphosphates were absent in the freeze-dried extracts, which instead contained pyrophosphate. The freeze-drying further resulted in lower fraction of orthophosphate monoesters, compared with rotary evaporation.

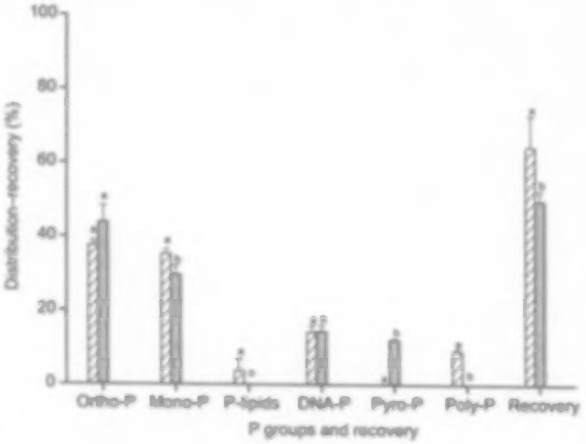
^{31}P NMR analysis

In the BD extracts from Lake Sønderby and Lake Erken, orthophosphate was the only P group detected (Fig. 2; Table 4). In Lake Kvie, the P_{BD} extract also contained orthophosphate monoesters (e.g., Turner et al. 2003) and P associated with deoxyribonucleic acids (DNA-P) (e.g., Turner et al. 2003).

In the NaOH extract from Lake Sønderby, orthophosphate, orthophosphate monoesters, P lipids, DNA-P, polyphosphate end groups – pyrophosphate, and polyphosphate middle groups (Hupfer et al. 1995; Makarov et al. 2002*b*; Turner et al. 2003) were identified. In Lake Erken, pyrophosphate and orthophosphate diesters, possibly in the form of teichoic acids (Teichoic-P) (Makarov et al. 2002*a*), were found in addition to the P groups observed in Lake Sønderby. No Teichoic-P, P lipids, or polyphosphates were found in the NaOH extract from Lake Kvie (Fig. 3; Table 4). Generally, orthophosphate was the dominating P group in the NaOH extracts, followed by orthophosphate monoesters, polyphosphates (when present), and DNA-P (Table 4).

In the Humic-P, the concentrations of P lipids, pyrophosphate, and polyphosphate middle groups were too low for quantification by ^{31}P NMR (Fig. 4; Table 4). Orthophosphate concentration in the Humic-P extracts was dramatically reduced compared with orthophosphate concentrations in the NaOH extracts. Orthophosphate monoesters were the dominant P group in the Humic-P, followed by DNA-P (Lake Sønderby, Lake Erken) or orthophosphate (Lake Kvie).

Fig. 1. Preconcentration of surface sediment from Lake Erken by rotary evaporation and freeze-drying. Hatched bars illustrate preconcentration by rotary evaporation; shaded bars illustrate preconcentration by freeze-drying. Groups of P compounds are quantified by ^{31}P NMR (nuclear magnetic resonance), while total recovery (Recovery) percentages are based on ICP (inductively coupled plasma) measurements. Error bars indicate the standard error of the mean. Different lower case letters indicate significant ($p < 0.05$) differences between the means of the P groups and extraction efficiency (*t* test). Ortho-P, orthophosphate; Mono-P, orthophosphate monoesters; Pyro-P, pyrophosphate; Poly-P, polyphosphate.



The results from the sediment trap material from Lake Erken revealed a decreasing trend in TP extracted, from the settling seston to the 15 m trap (Table 5; Fig. 5). Except for the lack of polyphosphates, the composition of P groups in the settling seston was similar to the P composition in the sediment. In general, the major changes in P group distribution appeared between the settling seston and the 8 m trap. One exception was the polyphosphates, which appeared in the 8 m trap and doubled their contribution to the total extracted P in the 15 m trap.

The distribution of the different P groups in the BD and NaOH extracts varied considerably among the lakes (Table 4; Fig. 6). Lake Kvie had a low fraction of pyrophosphate, polyphosphates, and orthophosphate diesters, but a high fraction of orthophosphate monoesters and orthophosphate. In Lake Erken, the sediment contained lower fractions of orthophosphate monoesters, but a higher fraction of polyphosphates and orthophosphate diesters (P lipids + Teichoic-P + DNA-P). The lowest fraction of orthophosphate monoesters was detected in Lake Sønderby, whereas intermediate fractions of polyphosphates and orthophosphate diesters were found.

Discussion

By combining ^{31}P NMR analysis and traditional P analysis on sequential extracts, a more elaborate description of the composition of nrP in the extracts was obtained, compared with the information given by the traditional P extraction

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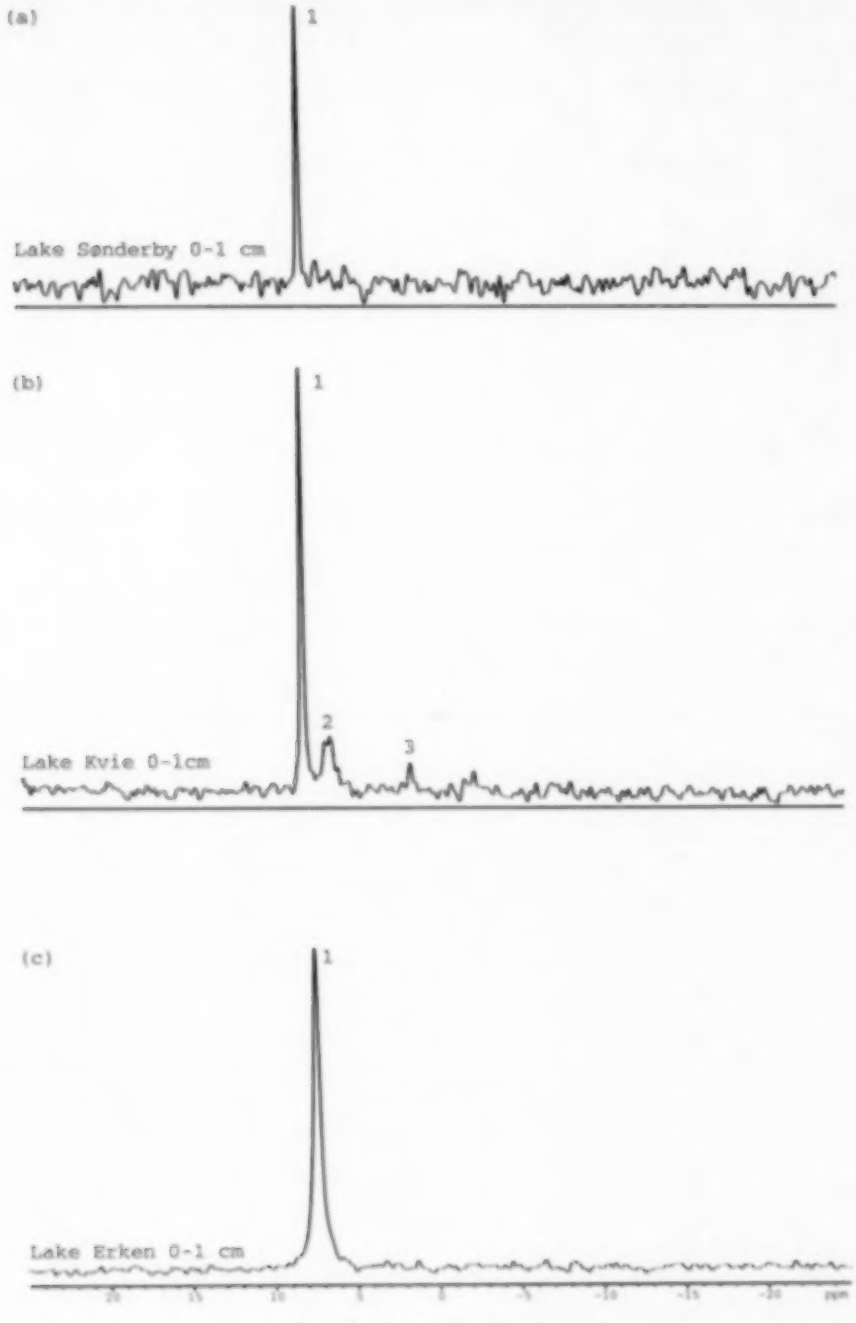
Fig. 2. ^{31}P NMR (nuclear magnetic resonance) analysis of the BD (bicarbonate dithionite) extracts from (*a*) Lake Sønderby, (*b*) Lake Kvie, and (*c*) Lake Erken. Chemical shifts (P compound groups) are identified by numbers: 1, orthophosphate; 2, orthophosphate monoesters; 3, DNA-P.



procedure. Thus, we found that the water, BD, and NaOH solutions extracted nrP, composed of several P groups, with a wide range of degradability, which will be discussed in more detail below. Our findings demonstrate that the nrP in general made up more than 15% of the sediment TP. Not all of the nrP was organic P, since inorganic polyphosphates and pyrophosphates were shown to make up a considerable proportion of the nrP.

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Table 4. Concentrations of total phosphorus (TP) in the extracts and percentages of the P groups in the extracts containing phosphorus not reacting with the molybdenum complex (nrP), determined by ^{31}P NMR spectroscopy.

	Lake Sønderby	Lake Erken	Lake Kvie
BD			
TP extracted (μmol·g^{-1} dry weight)	29	21	8
Orthophosphate (%)	100	100	63
Orthophosphate monoester (%)	0	0	25
Orthophosphate diester (DNA-P) (%)	0	0	12
NaOH			
TP extracted (μmol·g^{-1} dry weight)	80	28	37
Orthophosphate (%)	60	36	62
Orthophosphate monoester (%)	15	21	30
Orthophosphate diester			
Teichoic-P (%)	0	3	0
P lipids (%)	5	3	0
DNA-P (%)	6	11	5
Pyrophosphate (%)	9	11	3
Polyphosphate			
End group (%)	0	4	0
Middle group (%)	5	11	0
Humic-P			
TP extracted (μmol·g^{-1} dry weight)	9	7	10
Orthophosphate (%)	21	13	28
Orthophosphate monoester (%)	48	48	59
Orthophosphate diester			
Teichoic-P (%)	0	4	0
DNA-P (%)	31	26	13
Polyphosphate (end group) (%)	0	9	0

Note: BD, bicarbonate dithionite.

Preconcentration

Middle groups of polyphosphates were degraded to pyrophosphates when freeze-dried, indicating a possible underestimation of these when freeze-drying was used for preconcentration. Although not significant, there was a tendency for a higher distribution of orthophosphate in the freeze-dried extracts, indicating that some polyphosphates or even orthophosphate monoesters and P lipids were degraded to orthophosphate. The recovery of TP after preconcentration was significantly improved when rotary evaporation was used. Thus, our results show that rotary evaporation of the extracts is to be preferred to freeze-drying of the extracts, at least in Lake Erken. However, it is likely that the hydrolysis of polyphosphate could be reduced by introducing a pre-extraction in, e.g., EDTA, to chelate Ca^{2+}, which is known to catalyze the hydrolysis of polyphosphates (Van Wazer 1958).

BD extract

The orthophosphate signal in the BD extracts primarily reflects P dissolved from reduced Fe. In calcareous soils and sediments, some reactive P adsorbed onto the surface of $CaCO_3$ may also be extracted by the BD solution (e.g., Jensen et al. 1998; Golterman 2004), but this is hardly a problem in the three lake sediments investigated in this study. Vacuolar orthophosphate (Paytan et al. 2003) would probably also contribute to the orthophosphate signal and thereby lead to an underestimation of nrP determined from the ^{31}P NMR analysis when compared with the nrP determined according to Koroleff (1983). Hence, vacuolar orthophosphate is not detected by the molybdenum blue analysis, but is detected as orthophosphate by the ^{31}P NMR analysis.

Only orthophosphate was detected in the BD extract from Lake Sønderby, which was unexpected based on the high amount of nrP in this extract. Most likely, the P compounds constituting the nrP in the BD extract from Lake Sønderby were hydrolyzed to orthophosphate by the NaOH, which was added to obtain the ^{31}P NMR spectrum.

Apparently, the nrP extracted by the BD in Lake Kvie was more resistant to hydrolysis by NaOH than the nrP extracted in Lake Sønderby. Orthophosphate monoesters and DNA-P constituted the nrP in the BD extract from the dystrophic Lake Kvie and could represent P bound to humic acids, as mentioned in Golterman (2004). Furthermore, Baldwin (1996) found a strong correlation between rP extracted by $NaHCO_3$ and organic matter of humic origin. The finding of orthophosphate monoesters and orthophosphate diesters in the BD solution is not a surprise as, e.g., Makarov et al. (2002*a*) used $NaHCO_3$ to extract orthophosphate monoesters and orthophosphate diesters in soil samples. This implies that the BD solution, which is buffered by $NaHCO_3$, could do the same in sediments. However, based on our results, it is not clear whether the $NaHCO_3$ alone is responsible for the

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Fig. 3. ^{31}P NMR (nuclear magnetic resonance) analysis of the NaOH extracts from (*a*) Lake Sønderby, (*b*) Lake Kvie, and (*c*) Lake Erken. Chemical shifts (P compound groups) are identified by numbers: 1, orthophosphate; 2, orthophosphate monoesters; 3, teichoic acids; 4, P lipids; 5, DNA-P; 6, polyphosphate end groups; 7, pyrophosphate; 8, polyphosphate middle groups.

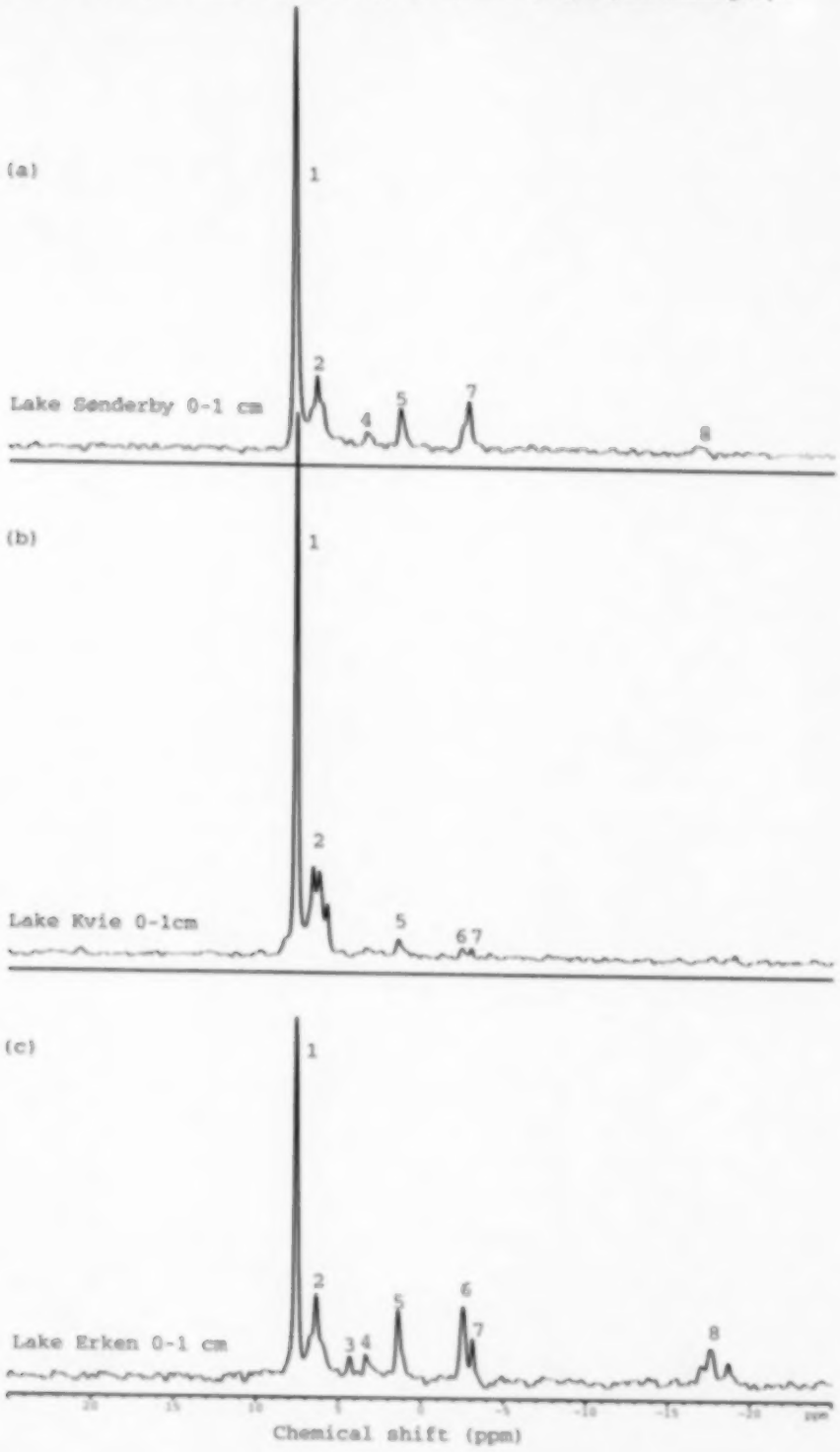

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Fig. 4. ^{31}P NMR (nuclear magnetic resonance) analysis of the Humic-P from (*a*) Lake Sønderby, (*b*) Lake Kvie, and (*c*) Lake Erken. Chemical shifts (P compound groups) are identified by numbers: 1, orthophosphate; 2, orthophosphate monoesters; 3, teichoic acids; 4, P lipids; 5, DNA-P; 6, polyphosphate end groups; 7, pyrophosphate; 8, polyphosphate middle groups.

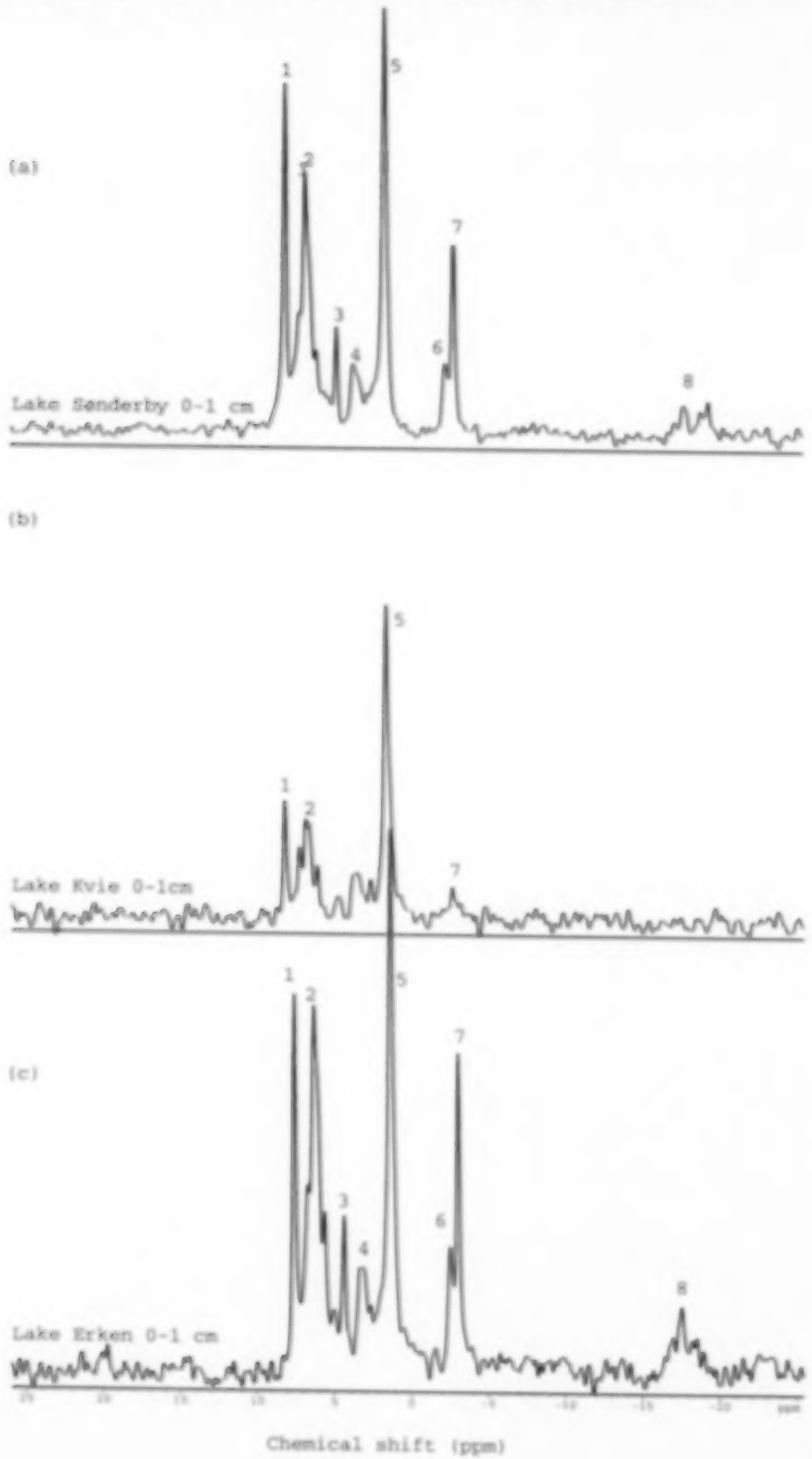

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Table 5. Total phosphorus (TP) of the NaOH extract and percentages of the P groups in the NaOH extracts from the settling seston and sediment traps in Lake Erken, determined by ^{31}P NMR spectroscopy.

	Settling seston	8 m trap	15 m trap
TP extracted (μmol·g^{-1} dry weight)	89.3	20.6	11.3
Orthophosphate (%)	36	30	20
Orthophosphate monoester (%)	30	13	13
Orthophosphate diester			
Teichoic-P (%)	1	2	2
P lipid (%)	8	4	4
DNA-P (%)	16	14	13
Pyrophosphate (%)	9	17	11
Polyphosphate			
End group (%)	0	9	20
Middle group (%)	0	11	17

extraction of nrP in the BD solution or if the dithionite dissolved some nrP as well.

NaOH extract

The highest variety of P groups, and by far the largest amount of nrP, was extracted in the NaOH extracts. As expected, high amounts of rP and orthophosphate was found in the NaOH extract from Lake Sønderby, probably reflecting the aluminium treatment in 2001. Also some vacuolar orthophosphate could contribute to the signal, as mentioned above.

Orthophosphate monoesters were the most important group of nrP in the NaOH extracts. The orthophosphate monoesters include mononucleotides, sugar phosphates, inositol phosphates, and P lipids (Paytan et al. 2003; Turner et al. 2003). Degradation products of, e.g., RNA (an orthophosphate diester), will be present in the orthophosphate monoesters as well, because of degradation in the NaOH extract (Makarov et al. 2002*b*; Turner et al. 2003). The dominance of orthophosphate monoesters in all three lakes makes this P group particularly interesting, as the NaOH-nrP pool in Lake Erken and Lake Sønderby was found to release P (e.g., Rydin 2000; Reitzel et al. 2005) during the summer periods. Furthermore, Ahlgren et al. (2005) showed that orthophosphate monoesters contribute to the sediment P release in Lake Erken. The fraction of orthophosphate monoesters increased with decreasing trophic status of the three lakes, indicating a reduced degradability or increased input of orthophosphate monoesters to the dystrophic Lake Kvie.

Interesting constituents of the orthophosphate monoesters in sediments and soils are the inositol phosphates (DeGroot and Golterman 1993; Suzumura and Kamatani 1995; Turner et al. 2002). These organic compounds with up to six P groups attached are known to exist in many lake sediments (DeGroot and Golterman 1993) and may be a major constituent of the organic P pool in soils and sediments (Turner et al. 2003). Turner et al. (2002) reported that inositol P plays a role in P storage, especially in plant seeds. Furthermore, Weimer and Armstrong (1979) showed that inositol P was naturally occurring in aquatic plants, which might constitute an important source of inositol P to the sediments.

Even though inositol P is chemically inert (Suzumura and Kamatani 1993), it degraded to soluble reactive P in marine sediments under anaerobic conditions (Suzumura and Kamatani 1995). These authors hypothesized that association of inositol P with Fe(III) protected it from microbial degradation under aerobic conditions. DeGroot and Golterman (1993) reported that inositol P formed insoluble salts with a variety of polyvalent cations and noted that inositol was strongly adsorbed to Fe(III). Even when Fe(III) was reduced, the inositol P remained adsorbed to Fe, which led the authors to conclude that inositol P might be highly stable in sediments. Celi and Barberis (in Turner et al. 2005) explained this stability of the Fe – inositol P complex by adsorption through four of the six P groups of inositol P onto the Fe(III) complex. This association results in a very stable complex, in which inositol P adsorbs stronger to Fe(III) than does phosphate (DeGroot and Golterman 1993; Celi in Turner et al. 2005).

Suzumura and Kamatani (1995) further suggested that inositol P was associated with humic acids in sediments, an association which might be mediated by Fe and Al (Paludan and Jensen 1995; Jensen et al. 2005). Thus, it is possible that the Fe-rich sediment in the dystrophic Lake Kvie combined with low pH and aerobic conditions favours a strong adsorption of inositol P in this sediment. This hypothesis could in fact explain the high proportion of orthophosphate monoesters found in Lake Kvie.

If inositol P can be mineralized to orthophosphate during anoxic periods in freshwater sediments, the anoxic P release, seen from many types of sediment, may not only be caused by the dissolution of Fe-P, as proposed by Mortimer (1941), or by bacterial release (Gächter and Meyer 1993), but also by release of P from inositol phosphates. However, the presence of highly reducing sulphides in marine sediments may explain possible differences in degradability of inositol P between marine and freshwater sediments because of interactions with Fe compounds known to form strong complexes with inositol P (Turner et al. 2005).

The amounts of P lipids, which are constituents of cell membranes, were in general low in all lakes and below the detection limit in Lake Kvie. The principle absence of P lipids in Lake Kvie may reflect a low density of bacteria in the sediment. The humic nature of Lake Kvie supports this suggestion, as the supply of organic P to the sediment is probably dominated by recalcitrant P compounds, rendering a low microbial activity, compared with Lake Sønderby and Lake Erken. This hypothesis is supported by the relatively high fraction of organic P in Lake Kvie sediment. However,

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Fig. 5. ^{31}P NMR (nuclear magnetic resonance) analysis of the NaOH extracts from the water column in Lake Erken: (*a*) settling seston, (*b*) 8 m trap, (*c*) 15 m trap. Chemical shifts (P compound groups) are identified by numbers: 1, orthophosphate; 2, orthophosphate monoesters; 3, teichoic acids; 4, P lipids; 5, DNA-P; 6, polyphosphate end groups; 7, pyrophosphate; 8, polyphosphate middle groups.

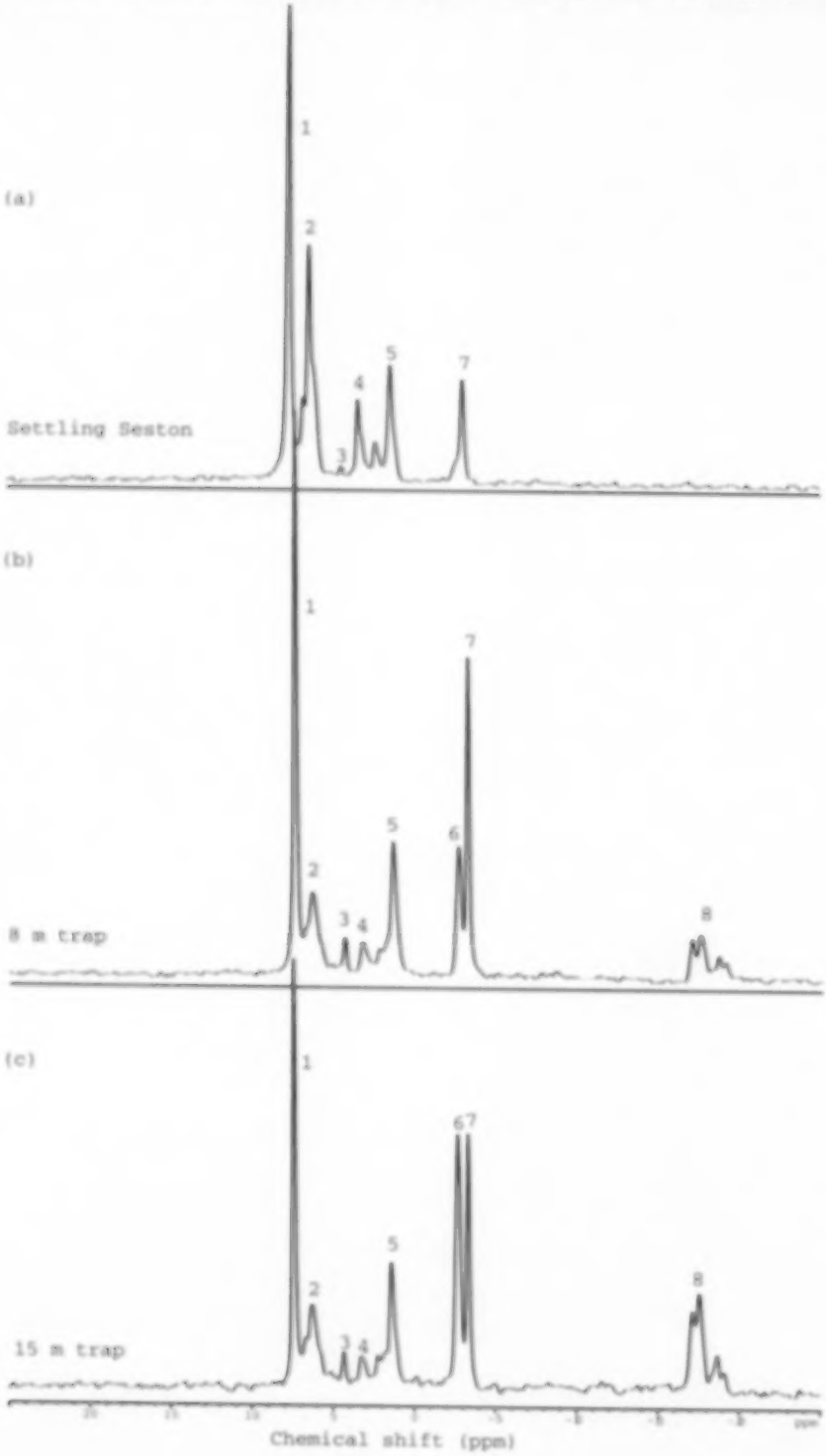

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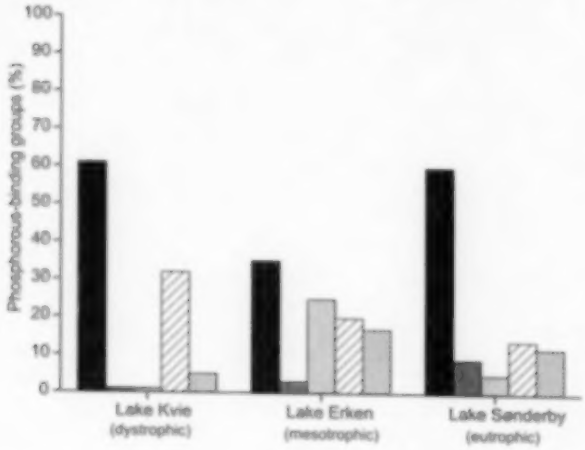
Fig. 6. Phosphorus-binding groups in the NaOH extract from the three lakes: solid bars, orthophosphate; dark grey bars, pyrophosphate; light grey bars, polyphosphates (middle groups and end groups); widely hatched bars, orthophosphate monoesters; densely hatched bars: orthophosphate diesters (Teichoic-P, P lipids, and DNA-P).



it is also possible that P lipids are underestimated in the hydrous NaOH extract, as these P compounds should be highly hydrophobic.

Since P lipids and DNA-P might reflect microbial activity and supply of autochthonous matter (such as for example phytoplankton), the higher amounts of P lipids and DNA-P in Lake Sønderby and Lake Erken sediments could then suggest more active sediments with a higher abundance of bacteria compared with Lake Kvie or a higher input from autochthonous material relative to Lake Kvie. From marine environments, it is known that DNA-P can be an important source of P to be recycled (Dell'Anno and Danovaro 2005). These authors found that the mineralization of extracellular DNA-P in the upper 10 cm of the deep-sea sediment accounted for 17% of the organic P mineralization. In lake sediments, less is know about the role of DNA-P in the overall P cycle, but Ahlgren et al. (2005) and Reitzel et al. (2006) demonstrated that DNA-P was mineralized faster than, e.g., orthophosphate monoesters.

Teichoic-P makes up another type of orthophosphate diester. Teichoic-P are found in Gram-positive bacteria (e.g., Condron et al. 1990; Makarov et al. 2002*a*), where they can constitute up to 85% of the total P in the cell wall. These sugar phosphates may not be detectable in P-limited environments, since bacteria can assembly their cell walls with teichoic acids free of P (Turner et al. 2003 and references therein) and furthermore use P bound in teichoic acids for growth under P-limiting conditions (Grant 1979).

The NaOH extracts from Lake Erken and Lake Sønderby contained considerable amounts of polyphosphates. These P-rich inorganic compounds are known to make up more than 20% of the weight in some bacteria (Davelaar 1993) and have recently been connected to the formation of phosphorite in marine environments (Schulz and Schulz 2005). Polyphosphates can be synthesized by algae, bacteria, and fungi, probably as a response to changing redox conditions, where polyphosphates are used as an energy source during anoxic conditions and synthesized during oxic conditions (Davelaar 1993). The finding of polyphosphates in the NaOH extract is in accordance with Törnblom and Rydin (1998), who found a correlation between bacterial P and the NaOH-nrP pool in Lake Erken.

As for the P lipids and the DNA-P, the low amount of polyphosphates in Lake Kvie probably reflects a low supply of labile organic matter from the water column and hence a low microbial activity, in contrast with Lake Erken and Lake Sønderby, which probably have higher input of polyphosphates from particulate organic matter in the water column and from the bacterial community. Along with polyphosphates in Lake Erken, pyrophosphate was also found. Presence of pyrophosphates and some orthophosphate may also be due to breakdown products of polyphosphates in the NaOH extract (Hupfer et al. 1995), and pyrophosphate has also been suggested to reflect human impact (e.g., fertilizers) in estuarine environments (Sundareshwar et al. 2001). This hypothesis is supported by our study, where we find the highest amount of polyphosphates in the eutrophic Lake Sønderby, which is the lake with the highest degree of human impact. Regardless of the origin of polyphosphates and pyrophosphates, several authors have documented the rapid mineralization of these P-rich compounds in lake sediments (Hupfer et al. 2004; Ahlgren et al. 2005; Reitzel et al. 2006), and as polyphosphates and pyrophosphates made up between 6% and 56% of the nrP, our results stress the importance of considering at least a part of the nrP as labile P directly involved in the sediment P release, as also suggested by Hupfer et al. (2004) and Ahlgren et al. (2005). Thus, it is possible that polyphosphates, which are believed to be released from bacteria at the same redox conditions where iron-bound P is dissolved, can make a major contribution to the internal P loading in many lakes.

The finding of polyphosphates in the NaOH extract and the possible hydrolysis to orthophosphate in extracts containing high amounts of Ca^{2+} (see Hupfer et al. 1995), known to catalyze the breakdown of polyphosphates (Van Wazer 1958), indicate that in ordinary P extraction schemes, some of the rP in the NaOH extract might originate from polyphosphates and thereby overestimate the amount of Al-P.

All of the P groups detected in the extracts from settling seston and sediment trap material were recovered in the surface sediment in Lake Erken. This indicates that the autochthonous P constitutes the dominant part of the P burial in Lake Erken. However, ^{31}P NMR analysis of the allochthonous P would have to be conducted to definitively exclude it as a source of sedimented P. The lack of polyphosphates in the settling seston may come as a surprise, but analysis of settling seston extracts collected in the spring showed that polyphosphates were abundant in the settling seston (data not shown). Thus, differences in species composition probably explain the lack of polyphosphates in the settling seston extract. The accumulation of polyphosphates in the 8 and 15 m traps most likely indicates colonization of the seston by polyphosphate-storing bacteria. Consequently, the surface sediment can be considered a transition zone influenced by the settling biogenic P from the phytoplankton, by bacteria in the water column, and by diagenetic processes in the sediment.

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Humic-P

The operationally defined Humic-P fraction contained substantial proportions of sediment TP in the three lakes. Jensen et al. (2005) found that Humic-P could constitute up to 50% of TP in North American lakes, and clearly, a better understanding of the composition and lability of such an important P fraction is required. In general, the Humic-P contained lower amounts of orthophosphate compared with the NaOH extracts, indicating that orthophosphate plays a minor role in humic acids and consequently illustrating that binding of orthophosphate through Fe and Al bridges in the humic matter is of minor importance, as demonstrated by Jensen et al. (2005). The orthophosphate signal may originate from vacuolar orthophosphate, or orthophosphate is released by degradation of more complex compounds when the precipitate was redissolved in NaOH.

Organic P (orthophosphate monoesters and DNA-P) was the major constituent of the Humic-P in all lakes. This distribution of P groups in the precipitate was also found by Bedrock et al. (1994) in humic extracts of peat soils, which might indicate a common origin of Humic-P. Only in Lake Erken did polyphosphates appear (but in low amounts). Together, this indicates that the Humic-P generally can be considered as a separation of organic P from inorganic P in the NaOH extract. Whether the precipitation of Humic P separates a more recalcitrant part of the NaOH extract can not be inferred from this study, although inositol phosphates, which may be associated with humic compounds, will give rise to an orthophosphate monoester signal as the one observed in the Humic-P. It should be stressed that even though Jensen et al. (2005) found that orthophosphate released during extraction was not precipitated as Humic-P, it is likely that the Humic-P included a mixture of smaller organic compounds extracted in the NaOH extract or was significantly altered during the preparation, e.g., by a previous BD treatment, as shown by Jensen et al. (2005). Furthermore, alterations of the Humic-P and the extracted nrP groups might have occurred during the relatively harsh extractions, as demonstrated by, e.g., Turner et al. (2003).

The existence of similar P compounds in different extracts should not be confused with the existence of similar P-binding groups, which are determined by the ^{31}P NMR analysis. In the case of similar P compounds in different extracts, this would imply that the sequential extraction scheme was not specific for extracting the particular P compound, and likely an additional extraction would be needed to recover the entire fraction of the given P compound. In the case of similar P compound groups in different extracts, the situation is different. Thus, different P compounds may be identified as similar P-binding groups by the ^{31}P NMR analysis. Hence, different P compounds such as inositol P and AMP would both be identified as orthophosphate monoesters by the ^{31}P NMR analysis. However, several parameters such as redox sensitivity, susceptibility to alkaline and acid hydrolysis, and the molecular configuration of the molecule surrounding the P nuclei determine the fate of the P compounds in the sequential P extraction procedure. Consequently, the existence of similar P-binding groups in different extracts should not be used to evaluate the specificity of the sequential P extraction scheme, and it should also be kept in mind that the sequential extraction schemes mostly aims on discriminating binding sites for reactive P.

In conclusion, ^{31}P NMR spectroscopy is a valuable tool for determining different organic P forms in aquatic particular matter. The distribution of the different organic P forms in surface sediment did vary substantially among lakes of different character, reflecting different catchment characteristics as well as trophic status. This different organic P composition among lakes will result in different availability to degradation and P turnover. A deeper understanding of organic P turnover in lakes will provide the tools for predicting lake ecosystem response to changes in, e.g., external nutrient load and climate change.

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Ecological differences between two closely related morphologically similar benthic whitefish (*Prosopium spilonotus* and *Prosopium abyssicola*) in an endemic whitefish complex

Benjamen M. Kennedy, Brett W. Thompson, and Chris Luecke

Abstract: Identifying the differences in ecology between closely related species occupying the same environment contributes to our understanding of community diversity, ecosystem structure, and species conservation. Endemic Bear Lake whitefish (*Prosopium abyssicola*) and Bonneville whitefish (*Prosopium spilonotus*) are benthic, morphologically similar, and closely related, yet the extent of differential resource use remains poorly understood. To determine the ecological differences between these two species, we studied their seasonal distribution and diet in Bear Lake, Utah–Idaho. We used bottom-set gill nets to examine how catch of each species of whitefish varied in relation to depth and season (spring and summer). In both spring and summer, Bonneville whitefish dominated the shallower depths (5–30 m), whereas Bear Lake whitefish dominated the deeper depths (45–55 m). Bonneville whitefish ate a variety of benthic invertebrates, but mostly Chironomidae, whereas Bear Lake whitefish fed mostly on Ostracoda. These data describe a closely related morphologically similar, yet ecologically distinct group of whitefish in an ecoregion completely different from those studied before. These results indicated that each species has a very different role in the Bear Lake ecosystem. To conserve this unique fish assemblage, both shallow and deepwater habitats need to be protected.

Résumé : L'identification des différences dans l'écologie d'espèces fortement apparentées qui vivent dans le même milieu contribue à la compréhension de la diversité des communautés, de la structure des écosystèmes et de la conservation des espèces. Les ménominis du lac Bear (*Prosopium abyssicola*) et les ménominis de Bonneville (*Prosopium spilonotus*) sont des poissons endémiques, benthiques, morphologiquement semblables et fortement apparentés; on connaît néanmoins mal leur utilisation différentielle des ressources. Afin de déterminer les différences écologiques entre ces deux espèces, nous avons étudié leur répartition saisonnière et leur régime alimentaire au lac Bear, Utah–Idaho. Des filets maillants installés sur le fond ont servi à montrer comment les captures de chacune des deux espèces de ménominis varient en fonction de la profondeur et de la saison (printemps et été). Tant au printemps qu'en été, les ménominis de Bonneville prédominent dans les profondeurs plus faibles (5–30 m), alors que les ménominis du lac Bear sont plus nombreux dans les eaux plus profondes (45–55 m). Les ménominis de Bonneville se nourrissent d'une variété d'invertébrés benthiques, mais surtout de Chironomidae, alors que les ménominis du lac Bear consomment principalement des Ostracoda. Ces données décrivent un groupe de ménominis proches parents à morphologie semblable, mais à écologie distincte, dans une écorégion totalement différente de celles étudiées antérieurement. Nos résultats indiquent que chacune des espèces a un rôle bien distinct dans l'écosystème du lac Bear. Les habitats, tant de faible que de grande profondeur, doivent donc être protégés afin de conserver cette association exceptionnelle de poissons.

[Traduit par la Rédaction]

Introduction

Quantitative data on the ecological differences between closely related species inhabiting the same environment can elucidate relationships between each species and their roles in the surrounding ecosystem, thus increasing our ability to create sound conservation plans (Scott and Angermeier 1998; Foster et al. 2003; Wood 2003). Ecologically similar species or groups that occupy analogous habitats and eat similar food items may have parallel habitat requirements and similar community roles and potentially compete for limiting resources. In contrast, ecologically different groups may have distinct requirements and diverse community roles and not compete for resources (Brandt et al. 1980; Crowder et al.

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1981). Most populations likely fall in between these extremes, so understanding the degree of ecological overlap is essential (Schoener 1970, 1974).

Fish communities occupying northern temperate lakes have populations with very diverse life histories and, in special cases, contain closely related sympatric ecotypes (Behnke 1972; Robinson and Wilson 1994; Taylor 1999). These populations include species in the families of Salmonidae (Bodaly et al. 1991; Snorrason et al. 1994), Gasterosteidae (Schluter 2000), and Osmeridae (Taylor and Bentzen 1993). Typically, populations of sympatric ecotypes contain a benthic ecotype and a limnetic ecotype (Lindsey 1963; Fenderson 1964; Bodaly 1979). The benthic ecotypes are relatively larger in body length and body depth and have fewer gill rakers that are shorter in length compared with limnetic ecotypes. These morphological differences are associated with differences in resource use (Bodaly et al. 1991; Bernatchez et al. 1999), where the benthic ecotype forages on the bottom and is more efficient at consuming benthic invertebrates compared with the limnetic ecotype, which forages in the open water and is more efficient at foraging on zooplankton (Schluter 2000). These populations are thought to occur as the result of resource-based divergent natural selection leading to reproductive isolation, and both sympatric speciation and allopatric speciation followed by secondary contact have been shown as modes of speciation (Bernatchez et al. 1996; Pigeon et al. 1997; Lu and Bernatchez 1999).

In the Bear Lake fish community, multiple sympatric species of whitefish exist; however, unlike most systems studied, the Bear Lake community contains two benthic species that are morphologically similar, as well as a limnetic species. Benthic Bear Lake whitefish (*Prosopium abyssicola*), benthic Bonneville whitefish (*P. spilonotus*), and limnetic Bonneville cisco (*P. gemmifer*) are closely related but are considered separate species (Bernatchez et al. 1991; Vuorinen et al. 1998). Genetic comparisons between Bonneville whitefish and Bonneville cisco showed that different alleles were present at two loci at large enough frequencies to indicate that they are very similar, diverging relatively recently (<35 000 years ago), but that gene flow does not occur (Vuorinen et al. 1998). Additionally, these two species are more related to *P. williamsoni* than to other members of the genus (Bernatchez et al. 1991). Furthermore, these species spawn at different times of the year, with Bonneville whitefish spawning in early December, Bonneville cisco spawning in early January, and Bear Lake whitefish spawning from mid-February to mid-March (White 1974; Thompson 2003; Albrecht 2004). Limnetic Bonneville cisco have been studied intensively and data indicate that they occupy the pelagic zone of the lake and eat zooplankton, and as in other systems with a limnetic ecotype, they have more gill rakers that are longer than those of the benthic species (White 1974; Luecke and Wurtsbaugh 1993; Bouwes and Luecke 1997). During these extensive studies on cisco, very few benthic whitefish or their prey were captured in the water column during any hour of the day during diurnal fish trawls, vertical gill net sets, or zooplankton tows.

Bear Lake and Bonneville whitefish are morphologically very similar and difficult to identify, and these factors have prevented ecological studies from being conducted. White (1974) found that when multiple characteristics were examined together, Bear Lake whitefish had a snout that was slightly more curved. However, Ward (2001) found these differences to be small. Fortunately, a recent morphological study developed and validated a key to quickly identify all size classes of each species in the field (Ward 2001), thus providing the opportunity for understanding the life history differences between each species and their roles in the ecosystem.

The objective of this study was to test the hypothesis that Bear Lake and Bonneville whitefish are ecologically separated by distribution and diet. Because these species have coexisted for thousands of years, we expected resource overlap to be low. We predicted that Bear Lake and Bonneville whitefish would have similar distributions but different diets or different distributions but similar diets or both different distributions and different diets. We examined how catches in gill nets of each species varied with depth and season and how diets varied between species and season. An information-theoretic approach was used to develop models of spatial distribution for both species in each season.

Materials and methods

Study area

Bear Lake is an oligotrophic lake located at 42°N, 111°20′W that straddles the northeast Utah – Idaho boarder (Lamarra et al. 1986; Wurtsbaugh and Hawkins 1990). At full pool, the lake covers 282 km^2 at an elevation of 1805 m above sea level. Bear Lake has a maximum depth of 62 m and a mean depth of 28 m. In approximately 3 out of every 4 years, the lake will ice over. The watershed geology consists mostly of limestone and other soft sedimentary rocks, causing the lake to be alkaline with calcite precipitation that strips phosphorus from the water. The substrate is dominated by fine marl sediments with limited areas of rock, sand, and snail shells from an extinct species of gastropod. Bear Lake is dimictic and cold and contains dissolved oxygen concentrations greater than 5 $mg \cdot L^{-1}$ throughout the water column throughout the year (Fig. 1). Epilimnetic midsummer chlorophyll *a* concentrations are near 0.5 $\mu g \cdot L^{-1}$, and zooplankton communities are dominated by the copepod *Epischura nevadiensis* and *Bosmina lacustris*, with *Daphnia pulex* and *Daphnia galeata* occasionally present. Benthic invertebrate biomass (0.34 g dry weight$\cdot m^{-2}$) is among the lowest recorded for midlatitude lakes (Wurtsbaugh and Hawkins 1990). The Bear Lake fish assemblage contains 13 fish species, including nine native species, four of which are endemic. The dominant piscivores in the system are native Bonneville cutthroat trout (*Oncorhynchus clarkii utah*) and nonnative lake trout (*Salvelinus namaycush*), both of which are maintained primarily by stocking. Currently, the top 6.5 m of water in Bear Lake is managed as an irrigation storage reservoir where water is diverted into the lake during spring and pumped out throughout the summer and fall. Typically the lake depth varies only about 1 m on a yearly scale but can rise or fall up to 5 m over the course of 5 to 10 years in association with extended drought or above-normal precipitation (Williams et al. 1962; Miller 1965; Laabs and Kaufman 2003).

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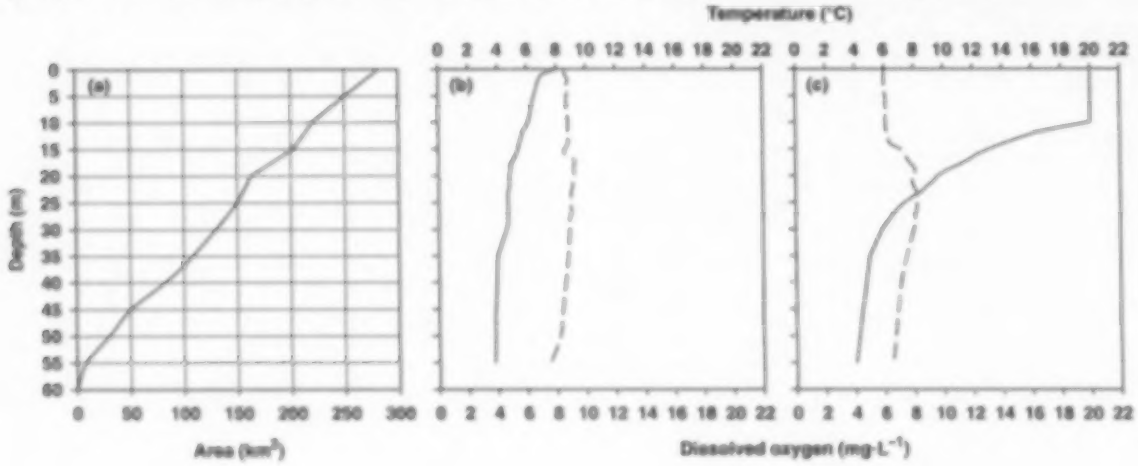
Fig. 1. (*a*) Hypsographic curve and (*b*–*c*) dissolved oxygen and temperature profiles of Bear Lake. Hypsographic curve is derived from Wurtsbaugh and Hawkins (1990). Each rectangle represents 0.25 km^3. Temperature measurements (solid lines) were taken in (*b*) May and (*c*) August of 2003. Dissolved oxygen measurements (broken lines) were taken in (*b*) May and (*c*) August of 2004.



Species identification

We classified whitefish as Bonneville whitefish or Bear Lake whitefish according to a key developed by Ward (2001). Ward (2001) used Bonneville whitefish caught in December and Bear Lake whitefish caught in February and progeny from both species reared in the laboratory to examine morphological differences between each species. He found that the two species differed at all size classes in the number of scales along the lateral line. Bonneville whitefish had 80 scales on average and never had less than 75 scales, whereas Bear Lake whitefish had 70 scales on average and never had more than 75 scales. Ward (2001) could correctly classify 99% of fish of all size classes by counting lateral line scales.

Spatial distribution and analyses

We measured whitefish distribution using the number of fish caught in gill nets set overnight. Gill net catch per net night (CPUE) was measured in July 2000, August 2000, 2001, 2002, and 2003, and May 2001, 2002, and 2003. This time frame enabled us to collect samples during summer stratification and near-spring isothermal conditions and in years when the lake was at full pool and when the lake was 5 m lower during drought conditions. For each sample period, we set experimental sinking gill nets for approximately 12–14 h from dusk to dawn to capture both crepuscular periods. In 2000 and 2001, we placed gill nets in a stratified random design to ensure sampling coverage throughout the entire area and depth of the lake. In 2002, gill nets were placed at depths of 15, 20, 25, 30, 35, and 40 m along four randomly chosen transects starting at the deepest part of the lake and extending to each shore (north, south, east, west). At depths of 50 and 55 m, gill nets were placed along two randomly selected transects from the original four (e.g., north and west). In May and August 2003, gill nets were placed at 5 and 10 m along the four transects. We used gill nets that consisted of 10 panels of different mesh size that were 4.6 m in length. The panels started at 1.27 cm stretch mesh and increased by 0.65 cm increments up to 5.08 cm stretch, then mesh size increased by 1.27 cm up to 8.89 cm. The numerous panels were included in an effort to catch a wide range of fish sizes.

We examined a set of candidate models in a model-averaging framework to describe whitefish distribution and estimate the mean and 95% confidence interval of CPUE for each species at each depth in spring and summer. An information-theoretic approach using Akaike's information criterion (AIC) works better than traditional statistical approaches in these situations because of the ability of AIC to simultaneously rank and select good approximating models that explain the data while balancing bias and precision (Burnham and Anderson 2002). In addition, model averaging using AIC avoids the disadvantages of using arbitrary α levels and comparing models two at a time. For example, the information-theoretic approach can provide an unbiased method to determine if a linear function is a more appropriate model for describing whitefish distributions than a log or polynomial function. Five models representing biological hypotheses about possible whitefish distributions in relation to depth were chosen as candidates (Table 1) to be simultaneously compared given our gill net data using AIC values with a small sample bias adjustment (AIC$_c$; Burnham and Anderson 2002). Models were ranked and compared using AIC$_c$ differences (ΔAIC$_c$) and normalized weights (w_i). The models with the lowest AIC$_c$ differences and the highest weights represent the best models for the data provided. Typically, more than one model may be appropriate, so to account for this model uncertainty and estimate distribution patterns more robustly, we used all models in a model-averaging framework. The final estimate of CPUE was the weighted average of CPUE from all models adjusted by how likely a given model represented the best model (w_i) so that the best approximating models contributed the most to the final estimate. Model-averaged 95% confidence intervals included model-selection uncertainties so that confidence intervals expanded if uncertainty existed in which models were best. All analyses were performed using a generalized linear model (PROC

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Table 1. Description of five a priori models used in model selection process to estimate model-averaged number of whitefish (*Prosopium spilonotus* and *P. abyssicola*) caught per gill net night across depths (m) found in Bear Lake in spring and summer, 2000–2003.

Model name	Hypothesis description	Structure
Random	Random relationship between number caught and depth	B_0
Linear	Number caught changes with depth at a constant rate	$B_0 + B_1(\text{depth})$
Pseudothreshold	Number caught changes with depth at a constant rate then approaches but does not reach an asymptote	$B_0 + B_1\ln(\text{depth})$
Flat then increasing or decreasing	Random relationship between number caught and depth then number caught changes with depth at a constant rate	$B_0 + B_1(\text{depth}^2)$
Third-order polynomial	Number caught reaches a maximal number at middle depths or number caught rapidly changes with depth	$B_0 + B_1(\text{depth}) + B_2(\text{depth}^2) + B_3(\text{depth}^3)$

GENMOD; SAS 2001) with a negative binomial link function to account for overdispersion in the count data (White and Bennetts 1996).

Diet collection and analyses

In 2002 and 2003, stomachs from each species were removed and preserved in 70% ethanol soon after capture. For both seasons, 10 fish between 100 and 300 mm TL (total length) of each species from each depth were randomly selected for diet analysis. Only Bonneville whitefish grow to a size larger than 300 mm TL, and individuals that large have been documented to become piscivorous and eat exclusively sculpin, so they were excluded from this study (Thompson 2003). Fish less than 100 mm TL were not caught in our nets. If 10 or fewer fish were caught at a given depth, then all the fish were used. In spring, all Bear Lake whitefish were used for diet analysis because of the small number of fish caught at that time.

Food items from each fish stomach were separated into groups and counted, and each individual item was measured (nearest 0.1 mm) with a dissecting microscope at 10× magnification. If a fish did not have any food items in its stomach, the fish was removed and a replacement was selected. Groups consisted of Chironomidae, Ostracoda, Pisidae, terrestrial (Homoptera and Hymenoptera), Oligochaeta, and Amphipoda. Length – dry weight equations from Wurtsbaugh and Hawkins (1990) were used to estimate dry weight biomass of each group. Head capsules of partially digested Chironomidae were measured at 30× and converted to undigested length using a regression equation (undigested length (mm) = head capsule width (mm) × 10.47 + 2.58, $r^2 = 0.67$, $p < 0.0001$) developed from a sample of 178 chironomids. The dry weight biomass for each food group contributing greater than 5% of the total diet was described between species, season, and their interaction with multivariate analysis of variance (MANOVA, PROC GLM; SAS 2001; $\alpha = 0.05$) and by visually assessing percentages of dry weights in stomachs. We then used logistic regression (PROC GENMOD; SAS 2001) to relate the presence or absence of each food group in a stomach to fish species, season, and fish length (TL). Separate analyses were performed for each food group. Logistic regression was used instead of linear regression because of highly nonnormal distributions of diet proportions. Estimates of effect size and precision were used to evaluate the importance of each factor.

Results

Spatial distribution

Bonneville and Bear Lake whitefish had very different benthic distributions in both spring and summer (Fig. 2; Table 2; Supplemental Table S1[4]). In spring, average Bonneville whitefish abundance was highest at 5 m and decreased gradually with depth to 35 m (Fig. 2; Supplemental Table S1[4]). Below 35 m, in the deep profundal zone, few Bonneville whitefish were caught. Bear Lake whitefish abundance had the opposite pattern. Few Bear Lake whitefish were caught until 40 m, and abundance increased rapidly from 45 m, with the highest abundance being at 55 m. Given the data, there were two models that were better than the others at describing Bonneville whitefish distribution and one model that best described Bear Lake whitefish distribution. These models were the flat then increasing or decreasing model and the polynomial model ($\Delta AIC_c < 2$, highest weights; Table 2). These models were characterized by properties that allowed for areas of abundance increase or decrease in addition to areas where abundance did not change. This contrasts with other models such as the linear model, in which abundance would have had to have changed at a constant rate throughout every depth, or the pseudothreshold model, which also assumed a constant change in abundance. The random model, which described a random relationship between numbers caught and depth, was ranked last, with a ΔAIC_c value greater than 10, indicating no support for this model.

In summer, Bonneville whitefish abundance was low at 5 m, then increased to a maximum at 15 m, and then decreased to 35 m. As in spring, few Bonneville whitefish were caught deeper than 35 m in the deep profundal zone (Fig. 2; Supplemental Table S1[4]). Unlike Bonneville whitefish, Bear Lake whitefish abundance had a similar pattern in summer as in spring. Bear Lake whitefish abundance was low until 40 m and then increased rapidly with depth to 55 m. The model that best described Bonneville whitefish and Bear Lake whitefish distribution in this season was the polynomial model (Table 2). The polynomial model fit the Bonneville whitefish distribution well because of its ability to describe the maximum levels of CPUE reached at middle depths, and

[4] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 5042. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/irm/unpub_e.shtml.

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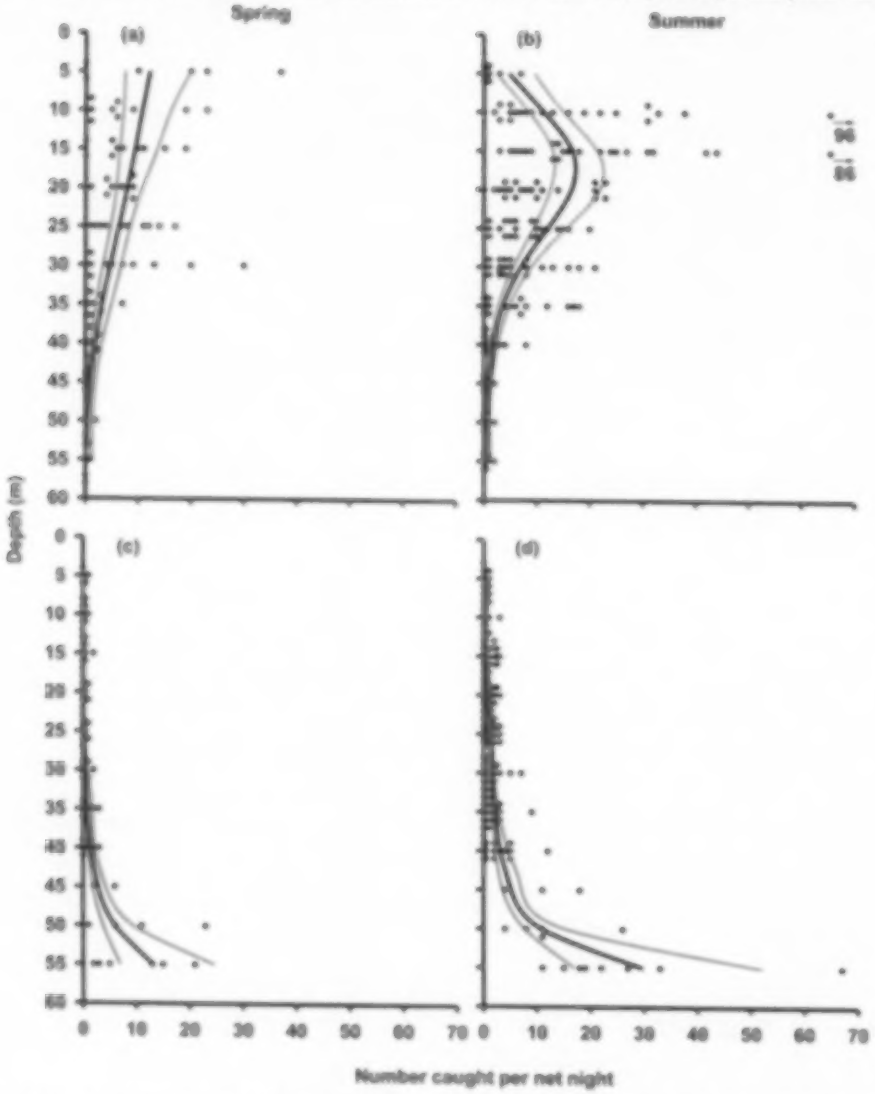
Fig. 2. Model-averaged mean and 95% confidence interval of the number of (*a* and *b*) Bonneville whitefish (*Prosopium spilonotus*) and (*c* and *d*) Bear Lake whitefish (*P. abyssicola*) caught per net night (CPUE) across depth for spring and summer, 2000–2003. Lines were created using predicted CPUE estimates for each 5 m depth interval and then point estimates were connected to each other to visually enhance the observed trends. Data points indicate actual numbers of whitefish caught on a given night and points are offset for clarity.



it fit the Bear Lake whitefish distribution well because of its ability to describe the rapid increase of CPUE at the deeper depths. The random model was again ranked last, with high ΔAIC_c values indicating no support for this model.

Diet

Associated with distribution differences were large diet differences between the species. Overall diet differed between Bonneville whitefish and Bear Lake whitefish (Pillai's trace = 0.39, $F_{[4,211]} = 82.36$, $p < 0.0001$) but did not differ across season (Pillai's trace = 0.97, $F_{[4,211]} = 1.68$, $p = 0.16$), nor was there an interaction between species and season (Pillai's trace = 0.98, $F_{[4,211]} = 1.11$, $p = 0.36$). In spring, Chironomidae, Ostracoda, Pisidae, and terrestrial insects contributed at least 5% each to the total diet of whitefish. Bonneville whitefish had all of these food items in their stomachs, but Chironomidae were the dominant food group followed by Pisidae. At a given depth, Chironomidae were present in most Bonneville stomachs (Supplemental Table S2[4]) and made up between 50% and 85% of the biomass on average for an individual fish (Fig. 3). Unlike Chironomidae, Pisidae were present in few stomachs, but they made up a high percentage of the biomass when present (Supplemental Table S2[4]). Ostracoda and terrestrials were present in few diets and made up small percentages of the diet when present (Supplemental Table S2[4]). In contrast to Bonneville whitefish, spring diets of Bear Lake whitefish consisted mostly of Ostracoda, with Chironomidae contributing to the weight in a couple of stom-

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Table 2. Ranking of a priori hypothesized models of whitefish (*Prosopium spilonotus* and *P. abyssicola*) distribution in Bear Lake in spring and summer, 2000–2003.

Species	Season	Model	K	N	AIC_c	ΔAIC_c	w_i
Bonneville whitefish	Spring	Flat then increasing or decreasing	3	83	−1229.10	0.00	0.56
		3rd polynomial	5	83	−1228.61	0.49	0.44
		Linear	3	83	−1220.11	8.99	0.01
		Pseudothreshold	3	83	−1208.56	20.54	0.00
		Random	2	83	−1192.78	36.32	0.00
	Summer	3rd polynomial	5	172	−5362.87	0.00	1.00
		Flat then increasing or decreasing	3	172	−5351.60	11.27	0.00
		Linear	3	172	−5337.61	25.26	0.00
		Pseudothreshold	3	172	−5313.24	49.63	0.00
		Random	2	172	−5286.92	75.94	0.00
Bear Lake whitefish	Spring	Flat then increasing or decreasing	3	83	−199.91	0.00	0.76
		3rd polynomial	5	83	−196.24	3.67	0.12
		Linear	3	83	−196.22	3.69	0.12
		Pseudothreshold	3	83	−182.57	17.34	0.00
		Random	2	83	−146.58	53.33	0.00
	Summer	3rd polynomial	5	172	−1070.70	0.00	0.81
		Flat then increasing or decreasing	3	172	−1067.70	3.00	0.18
		Linear	3	172	−1062.16	8.53	0.01
		Pseudothreshold	3	172	−1045.85	24.55	0.00
		Random	2	172	−943.54	127.16	0.00

Note: Models were ranked by Akaike's information criterion values adjusted for small sample bias (AIC_c) and AIC_c weights (w_i). Low ΔAIC_c and high AIC_c weights indicate best approximating models. K is the number of parameters (including the intercept) plus the dispersion factor (a parameter to measure dispersion of catch per net night data). N is the number of gill nets set. See Table 1 for model descriptions.

achs at shallower depths (Fig. 3). At the depths with the highest abundance (50 m and 55 m) of Bear Lake whitefish, Ostracoda averaged between 85% and 95% of their diet. Chironomidae were present in a small percentage, and Pisidae and terrestrial groups were found in very small percentages. At depths at which Bonneville and Bear Lake whitefish overlapped in low numbers (35 m and 40 m), diets were still different (Pillai's trace = 0.33, $F_{[4,57]} = 7.08$, $p < 0.0001$). However, a few Bear Lake whitefish diets were similar to those of Bonneville whitefish. Lastly, some Bear Lake whitefish collected at depths dominated by Bonneville whitefish had diets similar to those of Bonneville whitefish, whereas some were more like the diet of the majority of Bear Lake whitefish.

In summer, large diet differences continued to exist between species, and diets within a species were similar between seasons. In most Bonneville whitefish stomachs, Chironomidae continued to make up very high percentages of the biomass (Fig. 3), and Pisidae continued make up a high percentage of the biomass in some stomachs (Supplemental Table S2[4]). Also, Ostracoda and terrestrial insects were found at low percentages, similar to diet composition in spring. In contrast to Bonneville whitefish diet but again similar to spring diet, Bear Lake whitefish diets were dominated by Ostracoda at deeper depths. However, in this season, Ostracoda also dominated diets of the few Bear Lake whitefish found at depths with high abundances of Bonneville whitefish.

Diet — presence and absence

Fish species had a large effect on the presence or absence of a prey group, whereas season and fish length had lesser effects (Table 3). For most food groups, logistic regression models with species, season, and fish length effects fit the data well. For each model, the deviance divided by the degrees of freedom values were slightly less than one. The effect of fish species was large on the presence of Chironomidae, Ostracoda, and Pisidae. The odds of finding Chironomidae or Pisidae in Bonneville whitefish stomachs was 13 times higher than the odds of finding those items in Bear Lake whitefish (odds ratios from logistic regression). In contrast, the odds of finding Ostracoda in Bear Lake stomachs were 53 times higher than the odds of finding this item in Bonneville whitefish. The effect of season was important for Chironomidae and potentially important for Ostracoda, with these groups having a higher probability of being present in spring, although the 95% confidence interval for the season parameter estimate overlapped with zero in the Ostracoda model. The effect of length was important for Pisidae and terrestrial groups, with larger fish having a slightly higher probability of having these food groups present in their diet.

Discussion

Our results support the idea that the two benthic whitefish in the Bear Lake fish community are ecologically very different. First, these species inhabited different depths, with Bonneville whitefish dominating the upper strata and Bear Lake whitefish dominating the deep profundal zone. Second, these species had very different diets even at depths at which the species overlapped, with Bonneville whitefish eating a variety of benthic invertebrates, but mostly Chironomidae, and Bear Lake whitefish eating mostly Ostracoda. Additionally, Bonneville whitefish that reach sizes larger than 300 mm TL have been shown to eat exclusively sculpin, further expanding the differences between these species

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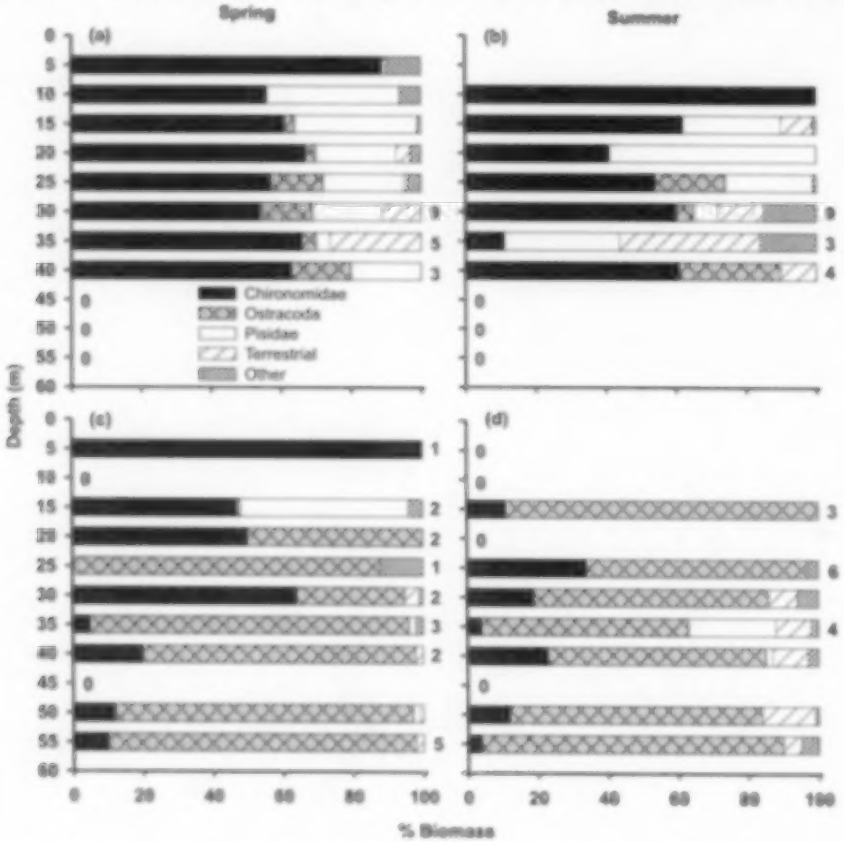
Fig. 3. Average percent of dry weight biomass of five prey items in spring and summer diets of (*a* and *b*) Bonneville whitefish (*Prosopium spilonotus*) and (*c* and *d*) Bear Lake whitefish (*P. abyssicola*) caught using gill nets at 5 m depth intervals in Bear Lake. At least 10 fish of each fish species were examined and averaged at each depth unless noted next to each bar. The "terrestrial" group consisted of mostly Homoptera and Hymenoptera. The "other" group consisted of mostly Amphipoda and Annelida.



(Thompson 2003). All of these patterns were evident in both spring and summer seasons. Although, CPUE of gill nets has been shown to vary with water temperature and fish body shape, which could bias our results, we think our results are robust to these effects. The two species in Bear Lake have almost the same body shape (Ward 2001), so their retention in the net should be equal. Additionally, differences in swimming performance in relation to water temperature are similar for closely related salmonids (Myrick and Cech 2000), and whitefish are known to be active in water temperatures as cold as 4 °C (Rudstam et al. 1994).

Given these distributions, Bonneville whitefish occupy a much larger benthic area than Bear Lake whitefish. Assuming Bonneville whitefish actively occupy depths of 5–30 m in spring and 10–30 m in summer, they would inhabit an area of 120 and 90 km^2, respectively, when the lake is at full pool (282 km^2). If Bear Lake whitefish actively occupy depths of 45–55 m, they would inhabit an area of only 50 km^2. At 5 m shallower than full pool, which can occur during a long string of drought years (5–10 years), Bonneville whitefish would occupy an area of in 110 km^2 in spring and 90 km^2 in summer and Bear Lake whitefish would occupy an area of only 30 km^2 in both seasons. This would be one-third less area than they occupied when the lake was at full pool.

Furthermore, these distributions indicate that Bonneville and Bear Lake whitefish occupy very different thermal regimes. In spring, Bonneville whitefish were associated with the highest temperature found in the lake (7 °C). In summer, high numbers of Bonneville whitefish were found within the fundamental thermal niche (14–18 °C) derived for *Coregonus* spp. in a laboratory setting (Magnuson et al. 1979; Christie and Regier 1988; Rudstam et al. 1994) and did not occupy the very shallowest depths with the warmest water temperatures (20 °C). In contrast, Bear Lake whitefish occupied the coldest water in the lake during both seasons (4–5 °C). Temperature is one of the most important factors affecting a fish's growth, survival, and reproduction, so fish that occupy different thermal regimes likely differ in many other life history characteristics, other than distribution and diet, making them even more ecologically different.

Because Bonneville and Bear Lake whitefish have such large differences in distribution and diet, they likely have very diverse roles in the Bear Lake ecosystem. Benthic fish are important in the structure and function of lake ecosystems, yet most research in lakes has focused on the pelagic

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Table 3. Parameter estimates and 95% log likelihood profile confidence intervals from logistic regression analysis of factors influencing the consumption of four prey items by whitefish (*Prosopium spilonotus* and *P. abyssicola*) in Bear Lake.

Prey item	Parameter	df	Estimate	SE	95% CI	χ^2	*p*
Chironomidae	Intercept	1	2.626	1.201	0.334 to 5.076	4.78	**0.029**
	Fish species	1	–2.568	0.459	–3.544 to –1.723	31.24	**0.001**
	Season	1	1.602	0.416	0.815 to 2.457	14.81	**0.001**
	Fish length	1	–0.002	0.006	–0.013 to 0.009	0.14	0.710
Ostracoda	Intercept	1	1.650	0.920	–3.500 to 0.131	3.22	0.073
	Fish species	1	3.967	0.561	2.979 to 5.229	49.97	**0.001**
	Season	1	0.701	0.379	–0.029 to 1.465	3.42	0.064
	Fish length	1	0.003	0.004	–0.006 to 0.113	0.38	0.537
Pisidae	Intercept	1	–3.598	1.011	–5.688 to –1.703	12.67	**0.001**
	Fish species	1	–2.537	0.510	–3.658 to –1.622	24.74	**0.001**
	Season	1	0.254	0.365	–0.459 to 0.980	0.48	0.487
	Fish length	1	0.015	0.005	0.006 to 0.025	9.51	**0.002**
Terrestrial	Intercept	1	–3.339	1.033	–5.453 to –1.386	10.45	**0.001**
	Fish species	1	–0.547	0.375	–1.304 to 0.174	2.13	0.145
	Season	1	–0.297	0.364	–1.020 to 0.413	0.67	0.415
	Fish length	1	0.011	0.005	0.002 to 0.021	4.95	**0.026**

Note: For the fish species parameter, positive values indicate a greater probability of the food item being present in Bear Lake whitefish and negative values indicate a greater probability of the food item being present in Bonneville whitefish. For the season parameter, positive values indicated a greater probability of the food item being present in spring. Bold text in the *p* column indicates significance at $\alpha = 0.05$ level. SE, standard error; CI, confidence interval.

open-water species. Recently, an effort has been made to reintegrate benthic processes back into our understanding of lakes (Vadeboncoeur et al. 2002). Fish that consume benthic invertebrates are important in this regard because they have been shown to alter the distribution, composition, and abundance of many benthic invertebrate species (Luecke 1990; Carlisle and Hawkins 1998). Also, many of these fish species are also prey for piscivores. Thus, they are important integrators of benthic and pelagic food webs (Vander Zanden and Vadeboncoeur 2002). The two whitefish in Bear Lake provide a unique example of this emerging field because of their unique upper and lower depth distributions and contrasting diets. The upper water habitat that Bonneville whitefish occupy in spring and summer is characterized by relatively higher amounts of food, more prey diversity, and higher densities of piscivorous fish (Wurtsbaugh and Hawkins 1990; Ruzycki and Wurtsbaugh 1999; Mazur and Beauchamp 2003). The high numbers of Bonneville whitefish found at these depths combined with diets of mostly Chironomidae and Pisidae and a high susceptibility to pelagic piscivores make Bonneville whitefish central to the upper strata community as an integrator of benthic and pelagic food webs. In contrast, the deepest habitats found in Bear Lake are characterized by low amounts of food, less prey diversity, and fewer piscivores. The high numbers of Bear Lake whitefish found at these depths and diets dominated by Ostracoda indicate a strong predator–prey relationship between Bear Lake whitefish and Ostracoda and a lesser relationship with piscivores.

Although ecological differences have been described for sympatric ecotypes of whitefish in both North America and Europe, these studies typically involved morphologically distinct whitefish in the genus *Coregonus* in recently glaciated lakes in very similar ecoregions. We provide quantitative evidence of sympatric ecotypes of benthic whitefish of the genus *Prosopium* in a lake not recently glaciated and in a different ecoregion, thus increasing the generality of sympatric ecotypes in the subfamily Coregoninae. Fishes of the genus *Coregonus* have been the focus of many population divergence and speciation studies (Todd et al. 1981; Bernatchez et al. 1996; Lu and Bernatchez 1999). Populations of these fishes in recently glaciated lakes (~11 000 years old) in the Holarctic ecoregion of northern North America and northern Europe have been shown to be both morphologically and ecologically diverse, but genetically very similar. As mentioned previously, these populations are usually characterized by a larger benthic ecotype and a smaller limnetic ecotype (Lindsey 1963; Fenderson 1964; Bodaly 1979). There is evidence that sympatric ecotypes also occur in *Prosopium* species and may be the result of adaptive radiation, but they have been studied much less (McCart 1970; Behnke 1972). Bear Lake, unlike many previously studied lakes, is in the arid Great Basin and is located south of other lakes containing sympatric ecotypes in North America by hundreds of kilometres. Additionally, Bear Lake is not recently glaciated. It has a long history (>35 000 years) of lake level fluctuations and connections with other large and now extinct lakes in the Great Basin (Miller 1965). Changes in lake level and connectedness were the result of climate associated with glaciation, earthquakes, and lava flows, but not glaciers directly. Furthermore, Bear Lake differs from many other lakes with sympatric pairs because unlike the usual one benthic – one limnetic ecotype, it contains two benthic ecotypes in addition to the limnetic ecotype (Bonneville cisco). Bonneville whitefish represent a shallower benthic ecotype that forages on a variety of invertebrates, and Bear Lake whitefish represent a deeper benthic ecotype that forages almost exclusively on Ostracoda. These data along with previously documented differences in genotype suggest that extending the generality of resource-based adaptive radiation in the subfamily Coregoninae to the *Prosopium* genus should be investigated. Additional studies on the evolutionary history of these species and examining if

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Bonneville whitefish can outcompete Bear Lake whitefish in shallower habitats and vice versa for deeper depths would clarify these ideas further.

Conservation implications

Bear Lake faces many potential threats, including nutrient loading, invasion of nonnative species, and stocking of piscivorous fishes. The distinct ecologies of each whitefish species in Bear Lake indicates that these threats will likely have different effects on each species, ultimately requiring complex conservation strategies. For example, increased nutrient loading could result in deoxygenation of the profundal zone and may have severe consequences for profundal Bear Lake whitefish by causing death or immigration into shallower areas. Boats from many areas throughout Utah and Idaho are launched daily onto Bear Lake throughout the summer. This could lead to nonnative benthic invertebrates becoming established, thereby changing the prey base on which these whitefish rely (sensu Kolar and Lodge 2002). Additionally, Bear Lake is stocked frequently with piscivorous native Bonneville cutthroat trout and nonnative lake trout, which could directly reduce the population size of one or both whitefish species or cause a change in habitat use (Ruzycki et al. 2001). A shift in either fish's distribution from any one of these threats may cause overlapping distributions and increased risk of competition and hybridization. Conservation of these fishes depends on conserving the diverse habitat that allows them to have different distributions (Lu and Bernatchez 1999). Studies examining how each of the threats might impact Bear Lake whitefish are needed to improve conservation strategies for these endemic fishes.

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The influence of siting and deterrence methods on seal predation at Atlantic salmon (*Salmo salar*) farms in Maine, 2001–2003

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Abstract: We document the nature and frequency of seal predation at Atlantic salmon (*Salmo salar*) farms in Maine and determine whether the severity of predation is related to the proximity of farms from one another and nearby harbor seal (*Phoca vitulina concolor*) haul-outs. We surveyed farm managers annually from 2001–2003 to document management techniques, husbandry practices, and predator deterrence methods employed for comparison with the extent of seal predation. Biweekly aerial surveys were conducted between January and March of each year to document harbor seal presence. An empirical estimate from a negative binomial model showed seal predation at farms declined significantly with distance to the nearest haul-out, suggesting that seal predation may be deterred by maximizing the distance between farms and seal haul-outs. Farms located further than 4 km from harbor seal haul-outs experienced minimal losses. At farms located within 4 km of harbor seal haul-outs, seal predation decreased with increasing distance from neighboring farms, indicating that areas where farms are concentrated may be more vulnerable. The regular replacement of primary and secondary cage netting was negatively correlated with seal predation. Finally, this study documents the apparent ineffectiveness of acoustic harassment devices at deterring seal predation.

Résumé : Nous étudions la nature et la fréquence de la prédation par les phoques dans les élevages de saumons atlantiques (*Salmo salar*) au Maine et déterminons la relation entre l'importance de la prédation et la proximité des élevages les uns avec les autres et avec les échoueries de phoques communs (*Phoca vitulina concolor*) des environs. À chaque année en 2001–2003, nous avons interrogé les gestionnaires des élevages afin de connaître leurs techniques d'aménagement, leurs pratiques zootechniques et leurs méthodes de dissuasion des prédateurs et de les relier à l'importance de la prédation par les phoques. Des inventaires aériens à toutes les deux semaines de janvier à mars de chaque année ont permis de déterminer la présence des phoques communs. Une estimation empirique dérivée d'un modèle binomial négatif montre que la prédation par les phoques dans les élevages décline significativement en fonction de la distance de l'échourie la plus proche, ce qui laisse penser que la prédation par les phoques pourrait être découragée en maximisant la distance entre les élevages et les échouries de phoques. Les élevages situés à plus de 4 km des échoueries de phoques communs connaissent des pertes minimales. Dans les élevages situés à moins de 4 km des échouries de phoques communs, la prédation diminue en fonction de la distance avec les élevages voisins, ce qui indique que les aires de concentration des élevages sont plus vulnérables. Il y a une corrélation négative entre le remplacement régulier des grilles primaires et secondaires des cages et la prédation par les phoques. Enfin, notre étude confirme l'inefficacité apparente des appareils de harcèlement acoustique comme moyens de dissuasion de la prédation par les phoques.

[Traduit par la Rédaction]

Introduction

Adverse interactions between seals and marine finfish farms are of economic and regulatory concern. The emergence of an aquaculture industry in the State of Maine has coincided with substantial increases in "regional seal populations" (National Marine Fisheries Service (NMFS) 1996).

Within the past 20 years, aquaculture in Maine has developed into a thriving shellfish farming industry and the lead producer of farmed Atlantic salmon (*Salmo salar*) in the United States. The culture and husbandry of marine finfish and shellfish is second only to lobsters in economic value for fisheries in the State (Maine Department of Marine Resources 1997). The total harvest of farm-raised finfish

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from Maine waters has risen from an initial 0.45 million kilograms in 1988 to a peak of over 16.33 million kilograms in 2000 (Jonathan Lewis, Maine Department of Marine Resources, P.O. Box 8, West Boothbay Harbor, ME 04575, USA, unpublished data).

High densities of fish concentrated in relatively small areas, such as net pens, inevitably appeal to fish-eating wildlife. Pinnipeds, in particular, exhibit plasticity in their feeding strategies and prey consumption, and individual seals and sea lions have learned to exploit situations where salmonids are concentrated and vulnerable (NMFS 1999). Offered protection under The Marine Mammal Protection Act of 1972, the harbor seal (*Phoca vitulina concolor*) population in Maine has been steadily increasing since 1981 (Kenney 1994; Marine Mammal Commission 1995; Gilbert and Guldager 1998). The population size from the latest survey in 2001 was estimated to be 99 000 seals, making them the most commonly found pinniped along the coast of Maine (Gilbert et al. 2005). Harbor seals are opportunistic feeders that prey on available species of demersal and pelagic fish and invertebrates, including salmonids (Bowen and Harrison 1996; Tollit et al. 1997; Williams 1999). They tend to be gregarious animals that haul-out in groups ranging from a few individuals to several hundred (Wynne and Schwartz 1999).

Maine waters are centrally located within the distribution of the western Atlantic subspecies of harbor seal. As a result, harbor seals can be found in the Gulf of Maine throughout the year; however, they are most abundant during late spring and summer. By early May, the majority of individuals have arrived in the sheltered bays and inlets of Maine. The greatest concentrations of harbor seals occur in Penobscot and Machias bays and off Mt. Desert and Swans islands. The majority of harbor seals migrate south to Long Island or the southern portion of the Gulf of Maine and southern New England in the fall (Payne and Selzer 1989; Gilbert et al. 2005; G.T. Waring, J.R. Gilbert, J. Loftin, and N. Cabana, unpublished data).

Seal predation can result in several types of serious negative impacts to the Maine finfish aquaculture industry. Seals are thought to bite and swipe at fish through the mesh netting, occasionally gaining access by creating holes in the netting. In addition to the loss of marketable product through direct injury and mortality, predation can result in stress, increased susceptibility to disease, and escapement of farm-raised fish (Pickering and Pottinger 1985; Pemberton and Shaughnessey 1993; Morris 1996). Besides the economic impacts of seal predation, potential escapements of farm-raised fish are of particular concern because of the endangered status of wild Atlantic salmon in Maine. On 17 November 2000, the Gulf of Maine distinct population segment of Atlantic salmon was listed as endangered under The Endangered Species Act of 1974 (ESA). Included in this listing were existing wild stocks from eight Maine rivers: the Dennys River, East Machias River, Machias River, Pleasant River, Narraguagus River, Cove Brook, Duck Trap River, and Sheepscot River (US Fish and Wildlife Service and National Marine Fisheries Service (USFWS & NMFS) 2002). The close proximity of salmon farms to five of the listed rivers has raised concern regarding the potential for competition, transmission of disease, and genetic mixing between escaped farmed salmon and wild stocks from the surrounding rivers (Peterson 1999; USFWS & NMFS 1999, 2002).

Currently, under state and federal discharge regulations, farms are required to develop site-specific predator deterrent plans (Maine Department of Environmental Protection, http://www.maine.gov/dep/blwq/docstand/aquaculture/MEG130000.pdf). With limited understanding of the interaction between seals and finfish aquaculture, the effectiveness of these predator deterrent plans is questionable. Investigations into the interactions between seals and finfish aquaculture sites throughout Maine will provide a better understanding of pinniped behavioral responses to the presence of this growing industry. In particular, knowledge of pinniped predation and response to deterrence methods employed is needed before effective guidelines and regulations for siting and containment can be developed.

The goal of this study was to document patterns of seal predation at marine salmon farms in Maine. The specific questions addressed include: Is seal predation at Maine marine salmon farms related to the proximity of farms from one another and nearby harbor seal haul-out sites? Are particular farm management practices related to levels of seal predation experienced at marine salmon farms in Maine?

Materials and methods

Study area

This study was conducted from 2001–2003 in Hancock and Washington counties, Maine. Data collection was restricted to the areas between Blue Hill Bay and Cobscook Bay, where all of Maine's marine salmon farms, at the time, were located (Fig. 1).

The coast in this region is generally rocky, with many bays and islands. Salmon farms are located in bays that provide protection from storms and adequate current to flush the sites of waste material. Small, half-tide ledges are abundant and are frequently used as haul-out sites by harbor seals for resting and pupping (Terhune and Almon 1983; Pauli and Terhune 1987; Kovacs et al. 1990). This means that suitable sites for aquaculture pens also provide desirable habitat for pinnipeds.

Maine finfish aquaculture industry questionnaires

To determine the nature and extent of seal predation, managers at marine salmon farms in Maine were sent annual questionnaires between 2001 and 2003. At the time of the study, there were a total of 43 marine salmon farms in Maine operated by four companies. Only 35 questionnaires, however, were mailed each of the three study years. Two of the 43 farms were known to have never been operational and were omitted from the study. In other instances, adjacent farms operated by the same company were often managed as a single farm and thus had to be treated as such for the purposes of this study. Farm managers within a company were often responsible for the operation of more than one farm. They were asked to fill out a separate questionnaire for each farm under their management.

Questions were modified from the approach of Ross (1988) and developed in cooperation with industry representatives (Salant and Dillman 1994). Farm managers were questioned regarding the following topics: farming practices, predation

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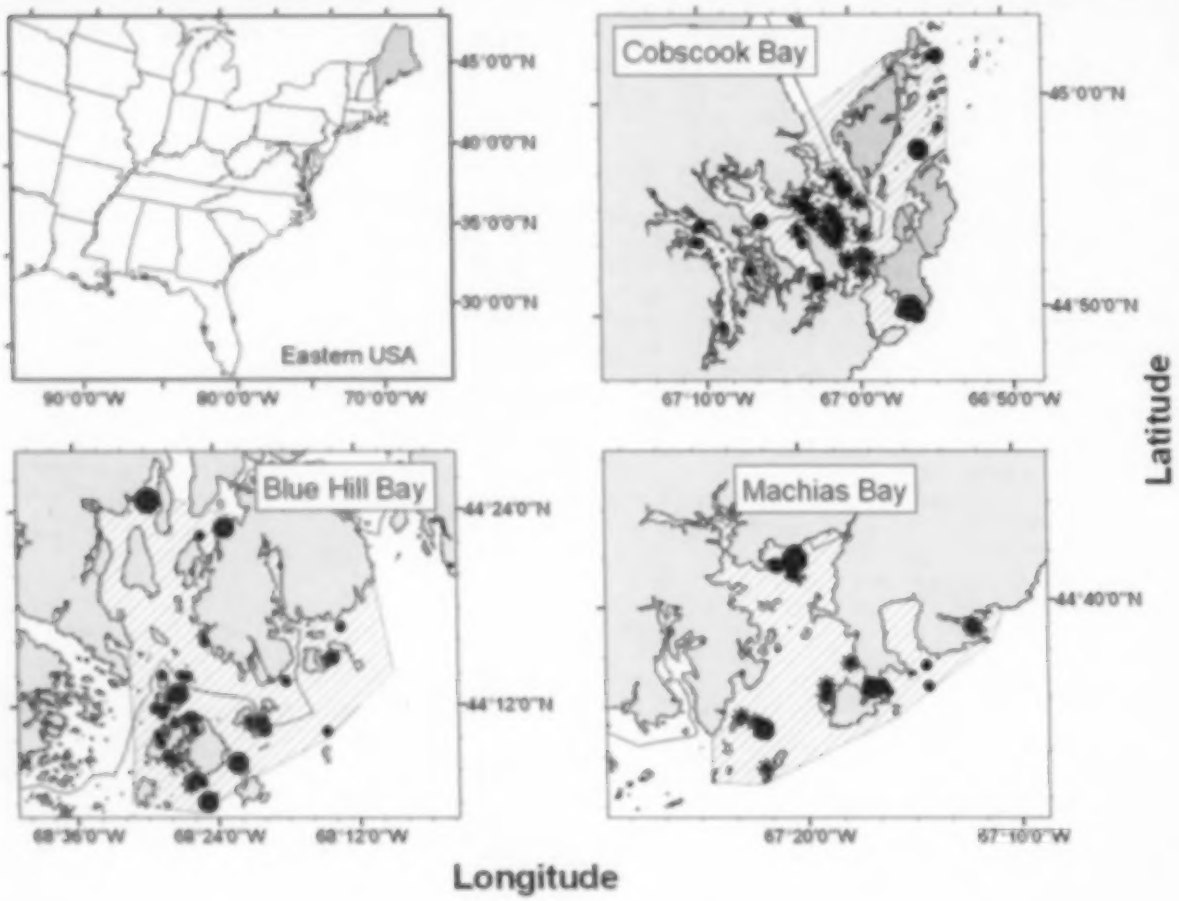
Fig. 1. Maps depicting locations of marine salmon farms along the Maine coast (asterisks), regions where aerial surveys of winter harbor seal (*Phoca vitulina concolor*) haul-outs were flown (hatched areas), and the average abundance of seals at nearby haul-outs (graduated solid polygons) between 2001 and 2003.



and predator deterrent methods employed, and their education and work experience in aquaculture-related fields. Each year, managers were asked to provide an estimate of the number of fish lost to seal predation. In addition to direct mortalities, fish that escaped through holes in the netting or were injured as a result of seal predation were also categorized as a loss. When there were no witnesses to a predation event(s), farm operators relied on indicators, such as bite marks, to determine which predator(s) were responsible for the damage. Many farmers also cited the difficulty inherent in differentiating between pre- and post-mortality predation by seals. Seals preying on live fish and mortalities are both, however, indicative of these predators having access to net pens and thus farm stock.

Respondents were asked to limit their answers to the previous year and the specific farm in question. Midpoints of any reported ranges were used in analyses. Farms that were fallow or non-operational were excluded from subsequent analyses. Farms were considered operational if occupied by fish at some point during the previous 12 months.

Aerial surveys and spatial analysis of winter harbor seal haul-out sites

Although actual numbers of seals within the region are greatest during the months of May through August, a task force convened to evaluate the interactions between pinnipeds and aquaculture in Maine reported that insurance claims submitted by Maine finfish growers increased during the winter months (Morris 1996; NMFS 1996). In Scotland, greater than 50% of all site managers reported that seal predation was highest during the winter months (Ross 1988). Results were similar in Australia: most seal attacks occurred between autumn and spring (Pemberton and Shaughnessey 1993). To document the winter distribution of seals around Maine marine salmon farms, aerial surveys were conducted from January through March of 2001, 2002, and 2003, the time of year when seal predation is thought to be most prevalent. Aerial surveys focused on the Blue Hill Bay, Machias Bay, and Cobscook Bay regions. Because of limited funding and the large geographical extent of the area, the region between Blue Hill Bay and Machias Bay was not surveyed.

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As a result, two farms (four lease sites) were omitted from aerial surveys (Fig. 1).

Aerial surveys were conducted 2 h either side of midday low tides and during favorable weather conditions to take advantage of the maximum number of harbor seals hauled out (Pitcher and McAllister 1981; Suryan and Harvey 1998; Simpkins et al. 2003). Because of poor weather conditions, surveys were conducted only three times in 2001 (3 February, 18 February, and 4 April). Surveys were flown on five dates in 2002 (22 January, 8 February, 22 February, 6 March, 26 March) and four dates in 2003 (26 January, 26 February, 11 March, 27 March). A Cessna 182 single-engine aircraft was used during all survey work. The plane surveyed at an altitude of approximately 305 m, dropping to approximately 213 m when photographing haul-outs. Haul-outs were photographed using a 35 mm camera with a 300 mm telephoto lens. Counts were made from 35 mm slides of surveyed haul-out sites. Occasional grey seals (*Halichoerus grypus*) were observed at surveyed haul-outs and were included in subsequent seal counts. The months during which aerial surveys were conducted, however, coincide with pupping and mating in grey seals. Thus, the majority of grey seals were likely at other, more suitable haul-outs (Reeves et al. 2002).

Distances between Maine marine salmon farms and documented seal haul-out sites or other marine finfish farms were calculated for each survey year using the Spatial Analyst extension in ArcMap Version 8.3 (ArcGIS, ESRI 2003). Distances were measured as a seal would be expected to travel from farm to farm or farm to haul-out, precluding measurements from being made across land (Pemberton and Shaughnessey 1993). The Maine Office of Geographic Information Systems provided maps of the Maine coastline and islands. The Maine Department of Marine Resources provided geographic locations of all marine finfish aquaculture facilities in Maine. The New Brunswick Department of Agriculture, Fisheries and Aquaculture provided the geographic locations of all finfish aquaculture sites in New Brunswick waters. Maps and farm polygons were converted to raster format with a 10 m^2 cell size. Because seals use small half-tide ledges, as well as islands, for hauling out, not all documented sites were represented in the same detail on available charts. Therefore, a 10 m^2 cell in the center of each haul-out was used for measurement purposes. Measurements were calculated from cell center to cell center. All haul-outs with a minimum of one seal on at least one occasion during a particular year were included in analyses.

Analysis procedures

To determine whether seal predation was related to the proximity of farms to harbor seal haul-out sites, a logistic regression analysis (SPSS Inc. 2000) was initially performed using a binary response variable for seal predation (0 = 100 or less fish lost to seal predation and 1 = greater than 100 fish lost to seal predation, for the year in question) and an independent continuous variable representing distance to the nearest harbor seal haul-out (km) for each farm (Agresti 1996).

Additional analyses were conducted in which a generalized linear model, using the negative binomial distribution for the random component, was used to analyze relationships between the magnitude of fish lost to or damaged by seal predation and explanatory variables representing proximity to nearby farms and harbor seal haul-out sites, farm operations, husbandry practices, predator deterrence efforts, and farm manager education and experience (Econometric Software, Inc. 2002). Because the estimated number of fish lost to seal predation was a discrete count, a generalized linear model assuming a Poisson distribution was initially used to evaluate the effect of each explanatory variable independently (Greene 2002). However, the distribution of losses had many zeros and a few large counts, which resulted in data that were overdispersed. A negative binomial distribution allowed for separate estimates of the variance and produced a better fit to the distribution of the estimated numbers of fish lost to seal predation (Liao 1994; Gabe and Hite 2003).

Dummy variables were created for all categorical explanatory variables. In this manner, the intercept stood in for the level of the excluded category and the significance tests of the remaining coefficients were comparisons to the missing category. For example, an independent variable with three categories was represented, in the model, by dummy variables for two of the three categories:

$$(1) \qquad Y = \alpha + \beta_1 D_1 + \beta_2 D_2$$

Testing the significance of β_1 and β_2 asked whether the categories identified by D_1 and D_2 were different from the omitted category (Neter et al. 1996).

Because of small sample sizes and collinearity between the explanatory variables, the equation was estimated using one explanatory variable at a time. A critical value of $p = 0.10$ was used in all statistical analyses because of limited power resulting from small sample sizes.

Results

Ninety-two percent of questionnaires were returned completed; responses were received from all but eight farms for a total of 97 returned questionnaires. A total of 40 farms were non-operational at some point during the 3-year study, primarily because of a disease outbreak in Cobscook Bay during 2001 (Jonathan Lewis, Maine Department of Marine Resources, P.O. Box 8, West Boothbay Harbor, ME 04575, USA, unpublished data). Additionally, three managers at active farms did not provide estimates of fish lost to seal predation. These limitations reduced the useable sample for analysis to 54 observations or farm-years. Each year that a farm was active represented one observation. Data were pooled across years because activities at a single farm often differ between years. For example, the number of fish stocked, the number and types of pens utilized, etc., can change from year to year.

Impacts of seal predation

Losses attributed to seal predation ranged from 0 to 27 629 salmon per farm per year, with 46% of farms reporting no losses. Seal predation on farm-raised salmon was slightly greater in 2001 than in 2002 ($n = 54$, $p = 0.104$). There was, however, no detected difference in seal predation levels between 2001 and 2003 ($n = 54$, $p = 0.189$) and 2002 and 2003 ($n = 54$, $p = 0.920$), further supporting the pooling of data across years (Table 1).

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Table 1. Estimates of the relationships between survey year and the number of fish lost to seal predation at Atlantic salmon (*Salmo salar*) farms in Maine.

Model[a]	Coefficient[b,c]	Marginal effect[d]	Frequency
Survey year vs 2003			
2001	1.589 (1.210)	4205	23
2002	–0.127 (1.253)	–335	19
Constant	6.919 (1.001)		
Survey year vs 2001			
2002	–1.715* (1.053)	–4540	19
2003	–1.589 (1.210)	–4205	12
Constant	8.507 (0.736)		

[a]All variables are binary and coded 1 if "yes", 0 if "no".
[b]Asterisks denote significance: ***, 1% level; **, 5% level; *, 10% level.
[c]Standard errors are listed in parentheses.
[d]The predicted change in the number of fish lost to seal predation following a one-unit increase in the explanatory variable X_i above the mean. For dummy variables, a one-unit increase in the explanatory variable is from a value of 0 (no) to 1 (yes).

Of all salmon losses to predation between 2001 and 2003, 84% were attributed to seals. Respondents at 48% of farms were of the opinion that seals were responsible for the greatest amount of predator-induced damage to farm stock, 33% considered birds to be the greatest problem, and 19% did not specify one group over another. The majority (74.3%) of respondents considered seal predation to be most prevalent during the months of January and February. Furthermore, market-size fish (3–5 kg) were reported, by 58% of respondents, to be the most affected by seal predation. Between 2001 and 2003, 40% of site managers felt seal predation had decreased from previous years, 8% felt predation was worse than in previous years, and 52% felt there was no difference.

Proximity to winter harbor seal haul-out sites

The probability of a salmon farm in Maine experiencing the loss of more than 100 fish to seal predation decreases significantly with increasing distance to the nearest harbor seal haul-out (Fig. 2) ($n = 46$, $p < 0.001$). Logistic regression analysis provided the following model:

$$(2) \qquad P(\text{>100 fish lost}) = \frac{\exp(1.23 - 0.60\text{MinDist})}{[1 + \exp(1.23 - 0.60\text{MinDist})]}$$

where MinDist represents the distance (km) to the nearest harbor seal haul-out, $\alpha = 1.23$ and $\beta_1 = -0.60$. For example, the probability of a farm experiencing seal predation if located 1 km from the nearest seal haul-out is 0.65, whereas the probability is only 0.24 if the farm is located 4 km from the nearest haul-out. The negative relationship between distance to the nearest harbor seal haul-out and the occurrence of seal predation at marine salmon farms was further supported by the likelihood ratio test ($-2(L_0 - L_1) = 15.60$, df = 1, $p < 0.001$). The Pearson chi-square goodness of fit test supported the null hypothesis of model fit (statistic = 37.04, $p = 0.76$, df = 44) (Agresti 1996).

Actual numbers of fish lost to seal predation also decreased with increasing distance to the nearest harbor seal haul-out site ($n = 46$, $p < 0.001$). According to the marginal effects resulting from negative binomial regression analysis, the predicted number of fish lost to seal predation decreases by 4053 fish for every 1 km increase above the average distance between a farm and the nearest harbor seal haul-out (Table 2). Maine salmon farms were located an average of 3.5 km from harbor seal haul-outs. The range of distances between active salmon farms and the nearest haul-out was 0.2–8.6 km. An examination of the raw data suggests that farms located further than 4 km from the nearest harbor seal haul-out site are likely to experience minimal losses of fish to seal predation. All farms located further than 4 km from the nearest harbor seal haul-out reported losses of less than 400 fish to seal predation. In fact, all but one of the 19 farms with no seal haul-outs within 4 km lost ≤100 fish to seal predation. Of those 19 farms, 15 reported no losses of fish to seal predation (Fig. 3).

Results from the previous analyses showed minimal losses of farm-raised fish to seal predation at farms located further than 4 km from the nearest haul-out. To determine what factors, other than proximity to seal haul-outs, were related to seal predation at Maine salmon farms, subsequent analyses were performed excluding observations at farms located further than 4 km from the nearest harbor seal haul-out ($n = 27$).

Abundance of seals at nearby winter haul-outs

The average minimum number of seals at nearby winter harbor seal haul-out sites was not related to levels of seal predation at marine salmon farms in Maine. Using negative binomial regression analysis, we determined that there was no relationship between the number of fish lost to seal predation and the total number of seals, averaged over the three survey years, at haul-outs within 4 km of a farm ($n = 49$, $p = 0.400$). For farms located within 4 km of a seal haul-out, we further determined that the average abundance of seals documented at the nearest haul-out site for each of the three survey years ($n = 27$, $p = 0.887$) and the weighted abundance of seals at the nearest haul-out (average abundance × (distance from farm)$^{-1}$) were not related to the magnitude of fish lost to seal predation ($n = 27$, $p = 0.873$) (Table 2).

Proximity to neighboring finfish aquaculture sites

The average distance between marine salmon farms was 2.1 km, with a range of 0.3 to 9.8 km. The results of this study suggest that farms located within 4 km of a harbor seal haul-out are likely to experience greater levels of seal predation with decreasing distance to the nearest neighboring salmon farm ($n = 27$, $p = 0.005$) (Table 2; Fig. 4). In other words, farms with harbor seal haul-outs nearby are more susceptible to seal predation if they also have another salmon farm(s) nearby.

Farm management

The results of models describing the relationships between farm management and the estimated number of fish lost to seal predation are presented (Table 3). The size of Maine farms, represented by the maximum number of fish on site at any one time during the year in question ($n = 27$, $p = 0.906$), and whether or not they were active (had fish on site) for the entire year in question ($n = 27$, $p = 0.370$) do not appear to

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Fig. 2. The predicted probability of seal predation (solid line) as a function of distance to the nearest winter harbor seal (*Phoca vitulina concolor*) haul-out. Observed losses (greater than 100 fish lost = 1) are denoted by diamonds.

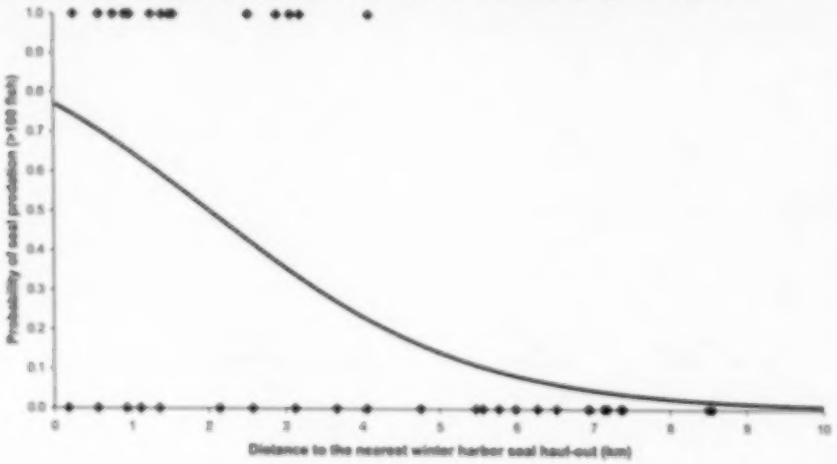

Table 2. Estimates of the relationships between proximity to other marine finfish farms or winter harbor seal (*Phoca vitulina concolor*) haul-out sites and the number of fish lost to seal predation at Atlantic salmon (*Salmo salar*) farms in Maine.

Model[a]	Coefficient[b,c]	Marginal effect[d]	Mean	Range
Distance to the nearest winter harbor seal haul-out (km)				
Distance	−1.032*** (0.208)	−4053	3.5	8.4
Constant	10.003 (0.903)			
Total number of seals within 4 km				
Sum of average number of seals at haul-outs within 4 km (2001–2003)	0.024 (0.028)	60	13.1	76
Constant	7.369 (0.683)			
Average abundance of seals at the nearest haul-out				
Average number of seals at nearest haul-out	−0.003 (0.020)	−11	15.1	110.3
Constant	8.297 (0.635)			
Weighted abundance of seals at the nearest haul-out				
Average number of seals at nearest haul-out × distance^{-1}	−0.007 (0.043)	−26	8.7	36.4
Constant	8.314 (0.673)			
Distance to the nearest neighboring farm (km)				
Nearest farm	−0.698*** (0.251)	−2860	2.1	9.5
Constant	9.139 (0.749)			

[a]Dummy variables are indicated with question marks and coded 1 if "yes", 0 if "no".
[b]Asterisks denote significance: ***, 1% level; **, 5% level; *, 10% level.
[c]Standard errors are listed in parentheses.
[d]The predicted change in the number of fish lost to seal predation following a one-unit increase in the explanatory variable X_i above the mean. For dummy variables, a one-unit increase in the explanatory variable is from a value of 0 (no) to 1 (yes).

be related to the estimated number of fish lost to seal predation. Between 2001 and 2003, Maine salmon farms stocked an average of 340 637 Atlantic salmon in net pens. Fifty-two percent of these farms had fish on site during the entire year in question, whereas the other 48% were devoid of fish at some point during the year.

The majority of site managers considered seal predation most prevalent during winter months. Of the farms located within 4 km of a haul-out site that did not have fish on site year-round, however, only five respondents included the months of the year that fish were on site. Thus, evaluation of a possible relationship between active months and seal predation was not possible.

Maine farmers reported using two types of pens for the culture of Atlantic salmon: steel cages and polar circles. Steel cages are comprised of square steel frames from which nets are suspended. They are usually arranged in systems of multiple pens separated by ~1.5 m walkways. Individual pens can be of varying sizes — Maine farmers reported using 12, 15, or 24 m^3 pens. Polar circles are constructed of high-density plastic frames from which nets are suspended. The Maine industry reported using polar circles with circumferences of 50, 70, and 100 m. Generally, polar circles are deployed individually within a grid system. Farms that used only one type of pen, either polar circles or steel cages, reported similar losses to seal predation ($n = 27$, $p = 0.889$).

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Fig. 3. The estimated number of fish lost to seal predation as a function of distance to the nearest winter harbor seal (*Phoca vitulina concolor*) haul-out.

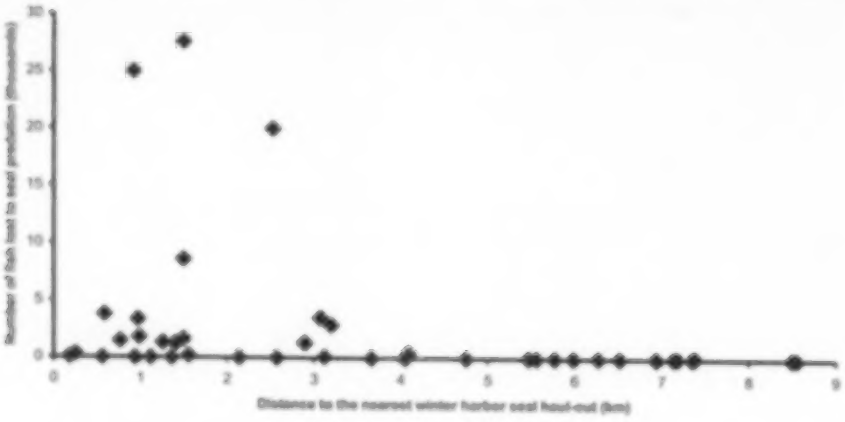
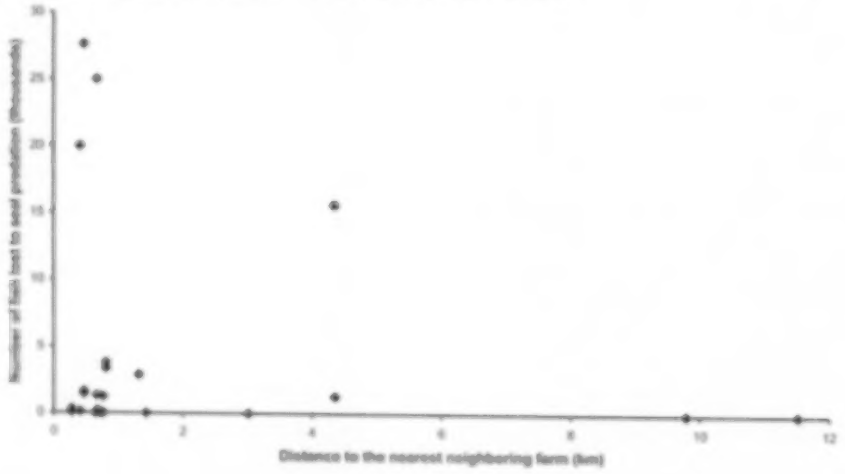


Fig. 4. The estimated number of fish lost to seal predation as a function of distance to the nearest other marine finfish farm (for farms located within 4 km of a winter harbor seal (*Phoca vitulina concolor*) haul-out site).



Farms that employed combinations of polar circles and steel cages, however, reported substantially greater losses of fish to seal predation than farms that used just polar circles (n = 27, p = 0.063) or steel cages (n = 27, p = 0.060) (Table 3).

The frequency with which fish mortalities were removed from net pens (n = 27, p = 0.340) was not found to be related to reported levels of seal predation. Managers reported the removal of fish mortalities from net pens once per week or more. Limited variability in mortality removal practices may have precluded the detection of a relationship between seal predation levels and this husbandry practice (Table 3).

The frequency with which the primary containment nets were replaced was one husbandry practice related to levels of seal predation experienced. Managers at farms where containment nets were replaced more than once per year reported fewer losses to seal predation than managers at farms where nets were replaced once per year or less (n = 27, p = 0.055) (Table 3).

Predator deterrent methods

The results of models describing relationships between predator deterrent methods and estimated losses of fish to seal predation are presented (Table 4). The deployment of acoustic harassment devices (AHDs) as a seal deterrent method at marine salmon farms appears to be ineffective. The estimated numbers of fish lost to or damaged by seal predation was greater at farms that employed AHDs than at farms where AHDs were not in use (n = 27, p = 0.100). The frequency of AHD use, however, was not related to seal predation. Reported losses of fish to seal predation were not statistically different between farms that used AHDs year-round and farms that employed them only seasonally (n = 18, p = 0.835). Nor was a relationship found between the number of hours per day that AHDs were in operation and reported numbers of fish lost to seal predation (n = 18, p = 0.185). Acoustic harassment devices were operated between 8 and 24 h per day (mean of 17.8 h·day^{-1}). Overall, only

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Table 3. Estimates of the relationships between variables describing farm operations and the number of fish lost to seal predation at Atlantic salmon (*Salmo salar*) farms in Maine.

Model[a]	Coefficient[b,c]	Marginal effect[d]	Frequency	Range
Farm size				
Maximum number of fish (active farms only)	0.000 (0.000)	0	NA	1 070 313
Constant	8.141 (1.120)			
Farm activity				
Were fish stocked at this farm all year?	0.899 (1.003)	3465		NA
Yes			13	
No			14	
Constant	7.695 (0.761)			
Pen type(s) used vs combinations				NA
Polar circles?	−2.256* (1.214)	−8700	12	
Steel cages?	−2.406* (1.280)	−9279	9	
Constant	9.465 (1.020)			
Pen type(s) used vs steel cages				NA
Polar circles?	0.150 (1.071)	579	12	
Combination of polar cirlces and steel cages?	2.406* (1.280)	9279	6	
Constant	7.059 (0.844)			
Frequency of mortality removal				NA
More than once a week?	−1.092 (1.143)	−4212		
Yes			7	
No			20	
Constant	8.447 (0.630)			
Frequency of containment net replacement				NA
More than once a year?	−2.048* (1.067)	−7899		
Yes			8	
No			19	
Constant	8.556 (0.628)			

[a]Dummy variables are indicated with question marks and coded 1 if "yes", 0 if "no".
[b]Asterisks denote significance: ***, 1% level; **, 5% level; *, 10% level.
[c]Standard errors are listed in parentheses.
[d]The predicted change in the number of fish lost to seal predation following a one-unit increase in the explanatory variable X_i above the mean. For dummy variables, a one-unit increase in the explanatory variable is from a value of 0 (no) to 1 (yes).

50% of farm managers felt that AHDs were fairly effective, whereas 44% believed they were ineffective, and only 6% believed they were completely effective ($n = 18$).

The deployment of secondary or predator nets around pens was also a commonly used form of predator deterrence. These nets are usually made of large-mesh, heavy nylon or polypropylene twine. There are several designs implemented by individual operations: single panels hung on the most vulnerable side of the pen; curtain nets that encircle the pen, yet have no bottom; and box nets that completely enclose the underwater portions of the fish pen. The types of nets used and methods of deployment were relatively standardized within the Maine industry. Because nearly all of the farms (26 of 27) used predator nets between 2001 and 2003, it was not possible to examine the relationship between predator net use and the estimated number of fish lost to seal predation. Also, because of limited variability in responses, statistical analyses could not be performed on the effects of predator net type, method of deployment, or frequency of use (year-round versus seasonally). Twenty-three of the 26 active farms within 4 km of a harbor seal haul-out site and employing predator nets between 2001 and 2003 (88%) reported using box-style nets. Additionally, 22 of 26 farms (85%) deployed predator nets around each individual pen versus around groups of pens or systems. Finally, 100% of managers reported using predator nets year-round rather than on a seasonal basis (Table 4).

Farms primarily differed in the achieved levels of separation between containment and predator nets and the frequency of predator net maintenance. The average level of separation between predator and containment nets was 1.6 m. Distances ranged from 0.6 to 2.4 m. The separation between predator and containment nets ($n = 26$, $p = 0.289$) was not related to levels of seal predation. There was, however, insufficient variability in net separation between farms and therefore limited ability to detect potential trends. The frequency with which predator nets were replaced was negatively correlated with levels of seal predation experienced ($n = 26$, $p = 0.001$). Farm managers who replaced predator nets more than once per year reported substantially fewer losses of fish to seal predation than managers who replaced the predator nets at their farms only once a year or less. Fifty-eight percent of managers at Maine salmon farms were of the opinion that predator nets were fairly effective, whereas 42% felt that they were completely effective ($n = 24$). None of the managers that interviewed felt that predator nets were ineffective at reducing seal predation (Table 4).

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Table 4. Estimates of the relationships between predator deterrent methods and the number of fish lost to seal predation at Atlantic salmon (*Salmo salar*) farms in Maine.

Model[a]	Coefficient[b,c]	Marginal effect[d]	Frequency	Range
Acoustic harrassment device (AHD) use				
Are AHDs used?	1.714* (1.041)	6 611		NA
Yes			18	
No			9	
Constant	6.862 (0.883)			
Frequency of AHD use yearly				
Are AHDs used year-round?	−0.168 (0.808)	−892		NA
Yes			12	
No			6	
Constant	8.686 (0.688)			
Frequency of AHD use				
Frequency of AHD use (h·day^{-1})	−0.079 (0.060)	−430	NA	16
Constant	9.849 (1.135)			
Separation between containment and predator nets (m)				
Net separation	1.929 (1.818)	9 732	NA	1.83
Constant	5.059 (2.957)			
Frequency of predator net replacement				
More than once a year?	−4.547*** (1.352)	−18 194		NA
Yes			4	
No			22	
Constant	8.459 (0.585)			

[a]Dummy variables are indicated with question marks and coded 1 if "yes", 0 if "no".
[b]Asterisks denote significance: ***, 1% level; **, 5% level; *, 10% level.
[c]Standard errors are listed in parentheses.
[d]The predicted change in the number of fish lost to seal predation following a one-unit increase in the explanatory variable *X*, above the mean. For dummy variables, a one-unit increase in the explanatory variable is from a value of 0 (no) to 1 (yes).

Discussion

Salmon farms attract a diverse array of predators (Ross 1988; Quick et al. 2004). Of the many predators that frequent marine finfish farms, pinnipeds are considered the most troublesome in other parts of the world, e.g., the western United States, Canada, Australia, and Scotland (Pemberton and Shaughnessey 1993; Nash et al. 2000; Quick et al. 2004). The results of this study have established the existence of a similar pattern in Maine. Between 2001 and 2003, there was a large variation in the estimated numbers of Maine farm-raised Atlantic salmon predated by seals. The majority of farm managers, however, considered seals to be the greatest predatory threat to farm stock.

The results of this study demonstrate that although seals may not specifically focus their foraging activities in areas where marine salmon farms are concentrated, they may take advantage of the available food source if it is located in close proximity to their haul-out sites. The probability of experiencing seal predation and the severity of that predation were negatively correlated with proximity to the nearest harbor seal haul-out. Farms with no harbor seal haul-outs within 4 km experienced minimal losses of fish attributed to seal predation. There were, however, farms with seal haul-out sites located less than 4 km away that also did not experience any seal predation. These results suggest that although distance to the nearest harbor seal haul-out site is not the only factor influencing seal predation at marine salmon farms, seal predation is substantially reduced at farms located further than 4 km from the nearest haul-out. Pemberton and Shaughnessey (1993) concluded that the severity of predation on farm-raised fish by Australian fur seals (*Arctocephalus pusillus*) is influenced by proximity to the nearest haul-out. A review by Nash et al. (2000) suggested that farms move offshore from haul-out sites and rookeries to mitigate predation impacts. Sepulveda and Oliva (2005) found no relationship between the magnitudes of fish losses at Chilean farms and distance to the nearest sea lion (*Otaria flavescens*) colony. Harbor seals, however, have significantly smaller foraging ranges than South American sea lions (Boulva and McLaren 1979; Campagna et al. as cited in Sepulveda and Oliva 2005) and may be more apt to take advantage of available food sources nearby, especially given the lack of wild stocks of fish available near shore during winter months. The results of our study suggest, however, that it does not matter how many seals are found at haul-outs within 4 km, providing evidence that a few individuals may repeatedly visit the pen sites.

At farms located near harbor seal haul-outs, seal predation was also linked to the separation between neighboring farms: areas where farms were concentrated (i.e., Cobscook Bay, Maine) appeared to be more vulnerable to seal predation.

The types of net pen(s) used on Maine salmon farms may influence levels of seal predation experienced. Those farms employing combinations of polar circles and steel cages reported significantly higher levels of seal predation than farms employing only a single pen type. The reasons for such a relationship are not entirely clear. This study found no difference in seal predation levels between farms using polar circles and farms using steel cages. It appears that no

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single pen type is better at protecting farm-raised salmon from seal predation. Further analysis of the influence of pen size and type on seal predation is warranted. This particular study examined pen type only and did not investigate such potential factors as stocking densities (number of fish·m^{-3}), numbers of pens on site, and pen layout. High stocking densities could likely increase predation losses because fish are more tightly packed and thus more exposed to the net sides and predating seals. Jamieson and Olesiuk (2001) found that seal predation levels at farms in British Columbia decreased when larger pens were installed.

Heavily fouled predator and containment nets are likely under greater stress and more vulnerable to tearing, allowing fish to escape or predators to enter the pens. Fouled nets also reduce water flow, likely having a negative effect on the health of the farm stock (Ross 1988; NMFS 1996). This study suggests that replacement of containment and predator nets more than once a year may result in decreased levels of seal predation. This is further supported by Sepulveda and Oliva (2005), in which Chilean farmers reported that predator nets were effective if well maintained, including cleaned every 6 months. Our conclusions are, however, limited by the fact that the replacement of nets once a year appears to be a relatively standard practice within the Maine industry.

This study strongly suggests the ineffectiveness of AHDs at deterring seal predation. Past studies suggest that AHDs function as a temporary means of predator deterrence only, citing habituation by seals (Jamieson and Olesiuk 2001; Quick et al. 2004; Sepulveda and Oliva 2005). Jamieson and Olesiuk (2001) reported that some of the largest losses of farm-raised fish to seal predation occurred while AHDs were in operation and that seal attacks did not decline until AHDs were phased out and larger pens were installed.

There is much controversy concerning the effects of AHDs on both target and non-target species. It has been suggested that AHDs lose their effectiveness because of potential hearing damage in seals (NMFS 1996; Johnston and Woodley 1998). This potential for hearing loss or damage in seals exposed to AHDs and uncertainty surrounding their effect on non-target species, such as harbor porpoise (*Phocoena phocoena*), other marine mammals, and particular species of fish, has prompted the prohibition of AHDs at marine finfish farms in British Columbia (Jamieson and Olesiuk 2001). Olesiuk et al. (2002) and Johnston (2002) suggest that harbor porpoises are deterred from passing through or inhabiting areas where AHDs are present. The use of AHDs on the west coast of Canada was also found to have a negative effect on occurrences and distributions of pacific white-sided dolphins (*Lagenorhyncus obliquidens*) (Morton 2000) and killer whales (*Orcinus orca*) (Morton and Symonds 2002). Additionally, herring and other clupeids may detect AHD signals, yet the resultant effects are not known at this time (NMFS 1996).

No relationship was found between the numbers of fish lost to seal predation and the reported separation between containment and predator nets. The range of net separations at Maine farms was, however, limited. Previous research suggests that insufficient separation between predator and containment nets leaves fish more accessible to seal predation (Pemberton and Shaughnessey 1993; NMFS 1996; Jamieson and Olesiuk 2001). Other studies have suggested the tensioning or weighting of nets to make them less pliable for predating pinnipeds (Quick et al. 2004; Sepulveda and Oliva 2005). Some Maine salmon farmers also cited the benefits of tensioning and (or) weighting nets to maintain their rigidity and prevent seals from pushing in on them and accessing the fish. Further study into the importance of proper net weighting and (or) tensioning within the Maine industry is warranted.

In conclusion, this study has highlighted some options for mitigating the potential impacts of seal predation at marine salmon farms in Maine, including establishing a minimum distance between farms and seal haul-outs and limiting concentrations of farms close to seal haul-outs. Additionally, results of this study suggest the importance of regular net cleaning and replacement. Further research into the relationship between frequency of fish mortality removal, pen size and type, and stocking densities is warranted. Finally, this study has demonstrated the ineffectiveness of using acoustic devices as methods of deterrence and the need for further investigations into the effects of AHDs on target and non-target species.

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The influence of prey availability on ontogenetic diet shifts of a juvenile piscivore

Tracy L. Galarowicz, Julie A. Adams, and David H. Wahl

Abstract: Young-of-year piscivores typically undergo ontogenetic diet shifts from planktivory to benthivory to piscivory. These shifts are often the result of changes in predator foraging abilities, but little is known about the influence of relative prey availability. As a result, we examined diet shifts across a range of sizes (20–150 mm) of a young-of-year piscivore, walleye (*Sander vitreus*), in feeding experiments in which zooplankton, benthic invertebrates, and fish were made available at different density combinations. Consumption of each prey type changed with walleye size and prey densities. Small juveniles (20 mm) selected zooplankton and fish, whereas larger walleye (40–100 mm) selected benthic invertebrates and fish. Relative prey densities influenced consumption; increased densities of more profitable prey types resulted in reduced consumption of less profitable prey. Walleye larger than 100 mm selected only fish. Foraging efficiencies also varied with size and prey types; small walleye (20 mm) were less likely to pursue benthic invertebrates and retain captured fish. These ontogenetic changes in foraging patterns are linked to prey profitability, have growth consequences for juvenile walleye, and have implications for understanding diet shifts of other juvenile fish.

Résumé : Durant leur première année, les piscivores subissent typiquement des changements de régime alimentaire qui passe de la planctonophagie à la benthophagie à l'ichtyophagie. Ces changements sont souvent le résultat de modifications dans les capacités de recherche de nourriture du prédateur, mais on sait peu de choses sur l'effet de la disponibilité des proies. Nous avons donc étudié les changements de régime alimentaire chez des jeunes piscivores de l'année, soit des dorés (*Sander vitreus*) de tailles variées (20–150 mm), dans des expériences d'alimentation dans lesquelles du zooplancton, des invertébrés benthiques et des poissons étaient disponibles en diverses combinaisons de densité. La consommation de chaque type de proie varie en fonction de la taille des dorés et de la densité des proies. Les jeunes de petite taille (20 mm) choisissent du zooplancton et des poissons, alors que les dorés plus grands (40–100 mm) sélectionnent des invertébrés benthiques et des poissons. Les densités relatives des proies influencent la consommation; des densités accrues de types de proies plus avantageuses causent une réduction de la consommation de proies moins profitables. Les dorés de taille supérieure à 100 mm choisissent seulement des poissons. L'efficacité de l'alimentation varie aussi en fonction des tailles des poissons et des types de proies; les petits dorés (20 mm) sont moins susceptibles de pourchasser les invertébrés benthiques et de retenir les poissons qu'ils capturent. Ces changements ontogéniques dans les patrons alimentaires sont reliés aux bénéfices associés aux proies, ils ont des conséquences sur la croissance des jeunes dorés et ils ont des implications sur notre compréhension des changements alimentaires chez d'autres jeunes poissons.

[Traduit par la Rédaction]

Introduction

Ontogenetic shifts in either resource or habitat use are commonly observed for invertebrates (Summers et al. 1997; Plaistow and Siva 1999), mammals (Adams 1996), reptiles (Young 1989; Rocha 1998), amphibians (Dupre and Petranka 1985), and both freshwater and marine fish (Osenberg et al. 1988; Dahlgren and Eggleston 2000; Hjelm et al. 2000). These shifts are the result of trade-offs between resource availability, competitive interactions, and predation risk that change with increasing body size (Werner and Gilliam 1984). For example, fish become more voracious predators and expand their breadth of diet items because of ontogenetic changes in mouth gape (Schael et al. 1991; Bremigan and Stein 1994), visual acuity (Breck and Gitter 1983), and swimming speed (Dabrowski 1989). However,

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prey availability is often changing concurrently, making it difficult to detect changes in foraging abilities.

Young-of-year (YOY) piscivores generally eat zooplankton as larvae, switching to benthic macroinvertebrates as juveniles and fish in later juvenile and adult stages (Mittelbach and Persson 1998). These ontogenetic patterns are often linked to optimal foraging theory, which assumes that foraging is the result of prey profitability and predator selection (Werner and Hall 1974; Charnov 1976). Energetic benefits of different prey types are the result of prey caloric density and size, as well as the ability of the predator to find, capture, and ingest prey (Mittelbach 1981). The efficiency of a predator in detecting and capturing prey is influenced not only by predator size, but also by availability of different prey types. For example, densities of zooplankton influence the timing of diet shifts from plankton to benthic macroinvertebrates for Eurasian perch (*Perca fluviatilis*) (Persson and Greenberg 1990) and bluegill (*Lepomis macrochirus*) (Werner et al. 1981). Prey densities can explain single shifts from either zooplankton to benthic invertebrates or benthic invertebrates to fish, but the conditions that determine use of multiple prey types by juvenile piscivores that undergo two ontogenetic diet shifts are not well known. Determining the effect of ontogenetic changes in predator foraging abilities and the influence of prey availability is a critical step in understanding these predator–prey relationships.

YOY walleye (*Sander vitreus*) are generally considered to undergo two ontogenetic diet shifts. Walleye are initially zooplanktivorous, switch to benthivory, and then become piscivorous (Maloney and Johnson 1957; Priegel 1970). However, as they increase in size, walleye do not necessarily remain strictly piscivorous; rather, zooplankton and benthic invertebrates may be included in diets over a range of sizes (Johnson et al. 1988; Kolar et al. 2003). In fact, when offered each prey type separately in the laboratory, juvenile walleye (20–100 mm) easily consume all three prey types (Galarowicz and Wahl 2005), but energetic consequences of piscine prey impart a growth advantage to YOY walleye (Galarowicz and Wahl 2005). Relatively few studies report both ontogenetic changes in diets of YOY walleye and prey abundance from the field (e.g., Fox 1989; Fox et al. 1989; Qin and Culver 1995), and little is known about how walleye foraging changes with size and the effects of differences in prey availability.

Because YOY piscivores such as juvenile walleye can consume multiple prey types but do not usually include all available prey types in their diet, we ask these questions. (*i*) What ontogenetic changes in consumption and selection occur when YOY piscivores are offered varying densities and taxa of prey? (*ii*) What mechanisms might be driving foraging choices for various fish sizes at different relative prey densities? To explore these questions, we conducted experiments varying the densities of each prey taxa to determine the influence of relative prey availability on ontogenetic changes in foraging by juvenile piscivores. These experiments provide insight into the influence of prey availability on YOY walleye ontogenetic diet shifts.

Materials and methods

The influence of body size and prey densities on foraging by walleye was examined in the laboratory by varying densities of zooplankton (*Daphnia* spp.), benthic invertebrates (chironomid larvae), and fish (larval carp, *Cyprinus carpio*, or bluegill) across a range of walleye sizes. Walleye (20, 40, 60, 80, 100, or 150 mm) were offered the three prey types simultaneously at different density combinations. Low and high densities were used for both zooplankton (low (2.5 L^{-1}) and high (25 L^{-1})), benthic invertebrates (low (25 m^{-2}) and high (500 m^{-2})), and fish (low (10 m^{-3}), and high (100 m^{-3})). An additional density of no fish (0 m^{-3}) was added for these prey. These treatments resulted in a total of 12 zooplankton – benthic invertebrate – fish combinations. The densities selected for each prey type were chosen because (*i*) they encompass the range found in natural systems for zooplankton (Welker et al. 1994; Claramunt and Wahl 2000), benthic invertebrates (Garvey and Stein 1998; Kline 2000), and fish (Claramunt and Wahl 2000), and (*ii*) they are below and above the inflection point of type II functional response curves for walleye offered each prey type separately (Galarowicz and Wahl 2005).

Walleye were obtained from the Jake Wolf Memorial Fish Hatchery, Illinois, as either larvae (9 mm) or juveniles (35 mm). Walleye were then held in the laboratory at the Kaskaskia Biological Station, Illinois Natural History Survey, Sullivan, Illinois, and grown to appropriate sizes. Walleye were offered all three prey types for at least 2 weeks before experimentation to ensure equal experience in experiments. Prey used in the experiments were collected from local ponds and reservoirs or cultured in the laboratory (see Galarowicz and Wahl 2005). Invertebrates used were from a narrow size range for daphnids (mean = 1.69 mm, standard error (SE) = 0.02) and chironomid larvae (mean = 11.35 mm, SE = 0.33). Invertebrate sizes used fell within the optimal range of sizes for juvenile walleye or similar-sized fish and were consistent with sizes available in the field (Mittelbach 1981; Mayer and Wahl 1997). Prey fish total length was maintained at optimal sizes of 25%–30% of the total walleye length (Einfalt and Wahl 1997). Bluegill were used as the prey fish, although larval carp were used for 20 mm size experiments because larval bluegill were not yet available. In addition, larval carp and bluegill are morphologically similar (Auer 1982) and have poorly developed antipredator behaviors.

We recorded consumption of each prey type by walleye feeding in experimental pools. Pool size (diameter range = 0.97–1.57 m) and volume (range = 0.11–0.98 m^3) were adjusted with walleye length to maintain consistent pool surface area – walleye length (20–25:1) and volume – walleye length (5–7:1) ratios. The pools were filled with filtered water and a sand substrate (1 cm in depth) and were aerated before each trial. Before initiation of a trial, walleye were starved at least 24 h to ensure similar hunger levels and effort in capture of prey. The three prey types were then added to the pool at one of the 12 prey density combinations. Both the zooplankton and benthic invertebrates were added at least 60 min before the start of the trial, whereas prey fish were introduced to the pool 15 min before a trial to allow acclimation. Before the start of a trial, an individual walleye was placed in an acclimation chamber within the pool for 60 min so that they were visually, chemically, and mechanically isolated from prey. At the start of the trial, the

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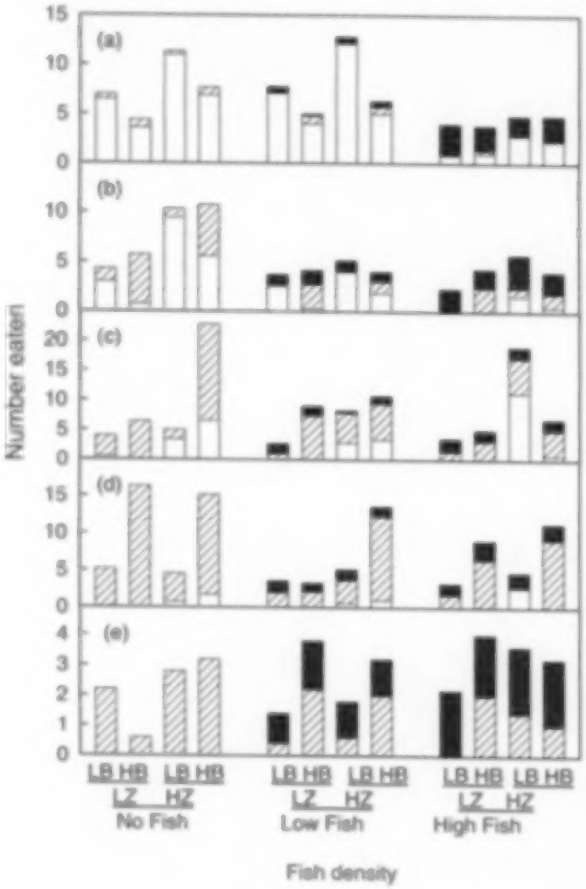
walleye was released into the pool, and the acclimation chamber was removed. The trial began when the walleye started swimming and searching for prey, and foraging behaviors were recorded by an observer for 30 min thereafter. At the end of the 30 min time period, the walleye were weighed (g) and measured (mm), and pool temperature (°C) (mean = 20.8 °C, SE = 0.07) was recorded. At least three replicates were conducted for each walleye size and zooplankton – benthic invertebrate – fish combination. Walleye were chosen randomly, selected from a large pool of walleye (N = 523), and were not used more than once for each treatment.

The number of orientations, pursuits, strikes, captures, and ingestions of each prey type, as well as the time at which behaviors occurred, were recorded (O'Brien 1979; Galarowicz and Wahl 2005). Consumption was defined as successful captures in which prey were not expelled (i.e., ingestions). The influence of prey densities on consumption of each prey type within and among all walleye sizes was examined with multivariate analysis of variance (MANOVA) with a sequential Bonferroni adjustment (Rice 1989). When significant multivariate effects were found, we then examined the univariate responses. Significant interactions were explored further by examining means determined through multiple comparisons methods. Data were log-transformed to homogenize variances (Zar 1999), although untransformed means (±1 SE) are presented graphically.

We examined the effects of density on prey selection using Manly–Chesson's alpha (α) selectivity index for constant prey populations, where $\alpha_i = (r_i/p_i)/\Sigma(r_i/p_i)$, r_i represents the proportion of prey eaten that is prey type i, and p_i represents the proportion of available prey that is prey type i (Chesson 1978; Krebs 1989). A selectivity index value greater than $1/n$ indicates selection for a prey type and a value less than $1/n$ indicates avoidance of a prey type, where n is the number of prey types available. In treatments in which fish were available, n = 3 and the critical value is $1/n$ = 0.33. In the no fish treatments, n = 2 and the critical value is $1/n$ = 0.50. Manly–Chesson's α represents the value of the prey in relation to its density and the other available prey types (Lechowicz 1982); the constant prey formulation was used because the number of prey eaten was generally less than 10 (Manly 1974; Krebs 1989).

Foraging efficiencies (pursuits per orientation, strikes per pursuit, captures per strike, and ingestions per capture) were also calculated for each prey type (Kolar and Wahl 1998). Behavioral probabilities were arcsin square root transformed, and differences among walleye size classes, prey types, and densities were determined using MANOVA with sequential Bonferroni adjustments (Rice 1989) followed by individual univariate analyses. We also examined the effect of walleye size and prey densities on captures occurring over the 30 min trial by combining the number of captures into six 5 min periods using a repeated-measures analysis of variance. In addition, we compared the time at which zooplankton, benthic invertebrates, and fish were first captured to test if the presence of alternate prey influenced initial capture time. Tukey's studentized range test was used to test for differences in capture time among prey types within a size class. All analyses were completed using PROC GLM (α = 0.05) in SAS (2001).

Fig. 1. Mean number of zooplankton (open bar), benthic invertebrates (hatched bar), and fish (solid bar) consumed by young-of-year walleye (*Sander vitreus*) in feeding experiments. Walleye of each size (*a*) 20, (*b*) 40, (*c*) 60, (*d*) 80, and (*e*) 100 mm) were offered all three prey types simultaneously at two densities of zooplankton (low (LZ) and high (HZ)), two densities of benthic invertebrates (low (LB) and high (HB)), and three densities of fish (none, low, and high).



Results

Prey consumption

The presence of alternate prey influenced ontogenetic patterns of consumption of zooplankton and benthic invertebrates but not fish (Fig. 1). The number of zooplankton eaten decreased as walleye increased in size, regardless of the treatment; in fact, the 100 mm and 150 mm walleye did not consume zooplankton. The number of zooplankton consumed was influenced by the density of zooplankton, as well as by the densities of benthic invertebrates and fish and the interaction of all three prey types. In contrast, consumption of chironomids increased with walleye size through 80 mm. Fish consumption increased slightly with size and fish density, but densities of the invertebrate prey types did not influence the number of fish eaten.

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Table 1. Effect of walleye (*Sander vitreus*) length and zooplankton (*Z*), benthic invertebrate (*B*), and fish (*F*) densities on consumption of each prey type.

		MANOVA		ANOVA						
				Zooplankton			Benthos		Fish	
	Source	*F* (df)	*P*	df	*F*	*P*	*F*	*P*	*F*	*P*
(*a*)	Model			3,87	4.77	0.004	5.17	0.003	24.81	0.0001
	Z	1.11 (3,74)	0.35							
	B	3.24 (3,74)	0.03	1,84	2.12	0.15	10.58	0.002	0.16	0.69
	F	8.96 (6,150)	0.001	2,84	5.55	0.005	2.98	0.06	37.22	0.0001
	Z × *B*	1.23 (3,74)	0.31							
	Z × *F*	0.21 (6,150)	0.97							
	B × *F*	0.68 (6,150)	0.67							
	Z × *B* × *F*	0.25 (6,150)	0.96							
(*b*)	Model			11,83	6.23	0.0001	8.95	0.0001	8.04	0.0001
	Z	6.23 (3,70)	0.0008	1,72	17.31	0.0001	2.27	0.14	0.01	0.91
	B	23.70 (3,70)	0.0001	1,72	11.41	0.001	58.56	0.0001	2.08	0.15
	F	15.15 (6,142)	0.0001	2,72	15.94	0.0001	12.42	0.0001	36.81	0.0001
	Z × *B*	0.74 (3,70)	0.53							
	Z × *F*	2.45 (6,142)	0.03	2,72	2.44	0.09	2.52	0.09	2.77	0.07
	B × *F*	0.35 (6,142)	0.91							
	Z × *B* × *F*	2.31 (6,142)	0.04	2,72	0.64	0.53	3.10	0.05	3.03	0.05
(*c*)	Model			11,84	19.32	0.0001	6.81	0.0001	9.25	0.0001
	Z	51.53 (3,71)	0.0001	1,73	144.56	0.0001	1.95	0.17	0.35	0.56
	B	11.89 (3,71)	0.0001	1,73	1.64	0.20	28.35	0.0001	2.09	0.15
	F	10.10 (6,144)	0.0001	2,73	0.27	0.76	3.80	0.03	46.15	0.0001
	Z × *B*	0.53 (3,71)	0.67							
	Z × *F*	1.07 (6,144)	0.38							
	B × *F*	6.56 (6,144)	0.0001	2,73	15.37	0.0001	6.76	0.002	0.63	0.54
	Z × *B* × *F*	7.90 (6,144)	0.0001	2,73	7.23	0.0001	10.89	0.0001	0.62	0.54
(*d*)	Model			8,83	2.88	0.008	8.64	0.0001	6.82	0.0001
	Z	6.85 (3,70)	0.0004	1,75	15.09	0.0002	3.78	0.06	2.35	0.13
	B	8.67 (3,70)	0.0001	1,75	0.12	0.73	27.24	0.0001	0.57	0.45
	F	6.89 (6,142)	0.0001	2,75	0.05	0.95	5.41	0.006	22.97	0.0001
	Z × *B*	0.23 (3,70)	0.87							
	Z × *F*	3.12 (6,142)	0.007	2,75	0.05	0.95	10.62	0.0001	1.11	0.34
	B × *F*	3.16 (6,142)	0.006	2,75	3.80	0.03	3.03	0.05	1.77	0.18
	Z × *B* × *F*	1.54 (6,142)	0.17							
(*e*)	Model			7,60	1.02	0.43	3.55	0.003	6.36	0.0001
	Z	1.84 (3,47)	0.15	1,53	1.03	0.31	2.11	0.15	2.91	0.08
	B	3.55 (3,47)	0.02	1,53	1.03	0.31	4.07	0.05	3.28	0.0001
	F	4.23 (6,96)	0.0008	2,53	1.05	0.36	1.52	0.23	13.28	0.01
	Z × *B*	3.27 (3,47)	0.03	1,53	1.03	0.31	1.75	0.19	7.21	0.11
	Z × *F*	3.37 (6,96)	0.005	2,53	1.05	0.36	7.10	0.002	2.34	0.09
	B × *F*	1.71 (6,96)	0.13							
	Z × *B* × *F*	1.34 (6,96)	0.23							

Note: Walleye (20–150 mm) were fed zooplankton, benthic invertebrates, and fish simultaneously in pool experiments at all combinations of low densities, high densities, and no fish treatments. Multivariate analysis of variance (MANOVA) tests of effects of walleye length and prey density and analysis of variance (ANOVA) tests on consumption of each prey type for significant terms in MANOVA are presented for (*a*) 20 mm, (*b*) 40 mm, (*c*) 60 mm, (*d*) 80 mm, and (*e*) 100 mm walleye.

Within each size class, we also found differences in consumption among prey types. The number of zooplankton consumed by 20 mm walleye was only influenced by the density of fish prey (Table 1*a*). In the high fish density treatments, consumption of zooplankton was approximately four times less than with both the low fish and no fish densities (Fig. 1). Consumption of benthic invertebrates and fish were significantly influenced by their respective densities (Table 1*a*). More chironomids were consumed (approximately three times more) in the high benthos density treatments, and five times more fish were consumed in the high rather than the low fish treatments (Fig. 1).

The importance of alternate prey densities on consumption was also evident for the 40 mm walleye (Table 1*b*). Consumption of zooplankton increased with zooplankton density and decreased with both higher benthic invertebrate and fish densities (Fig. 1). The number of benthic invertebrates consumed increased with benthos density but declined

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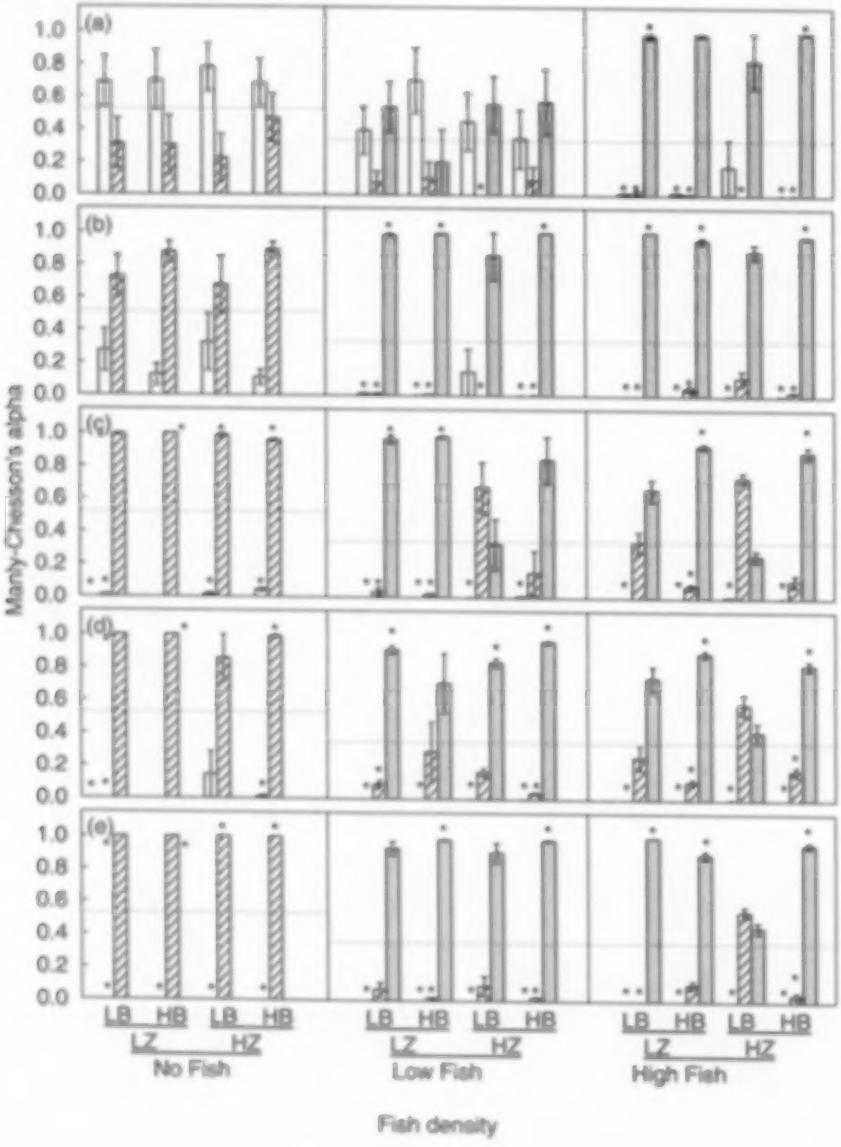
Fig. 2. Mean (±1 standard error) values of Manly–Chesson's α selectivity index of zooplankton (open bars), benthic invertebrates (hatched bars), and fish (shaded bars) consumed by (*a*) 20, (*b*) 40, (*c*) 60, (*d*) 80, and (*e*) 100 mm walleye during feeding experiments. Walleye (*Sander vitreus*) were offered all three prey types simultaneously at two densities of zooplankton (low (LZ) and high (HZ)), two densities of benthic invertebrates (low (LB) and high (HB)), and three densities of fish (none, low, and high). Horizontal dotted lines represent the critical value; α values greater than the critical value indicate positive selection, whereas α values less than the critical value indicate negative selection. Asterisks indicate values significantly different from random feeding (*t* test with Bonferroni adjustment to insure overall error rate of 0.05).



dramatically when fish were available (Fig. 1; Table 1*b*). Fewer chironomids were consumed when more zooplankton was available. Fish consumption increased with fish density (Table 1*b*).

Consumption by both the 60 and 80 mm size groups was influenced by all three prey densities and interactions between benthos and fish densities (Tables 1*c* and 1*d*). Additionally, consumption by the 60 mm walleye was influenced by an interaction among all three prey types, and the 80 mm walleye were influenced by an interaction between the invertebrate prey (Tables 1*c* and 1*d*). Zooplankton were primarily consumed in the high zooplankton treatments but only when fish were not available (Fig. 1). The exception was for 60 mm walleye with high fish densities that also

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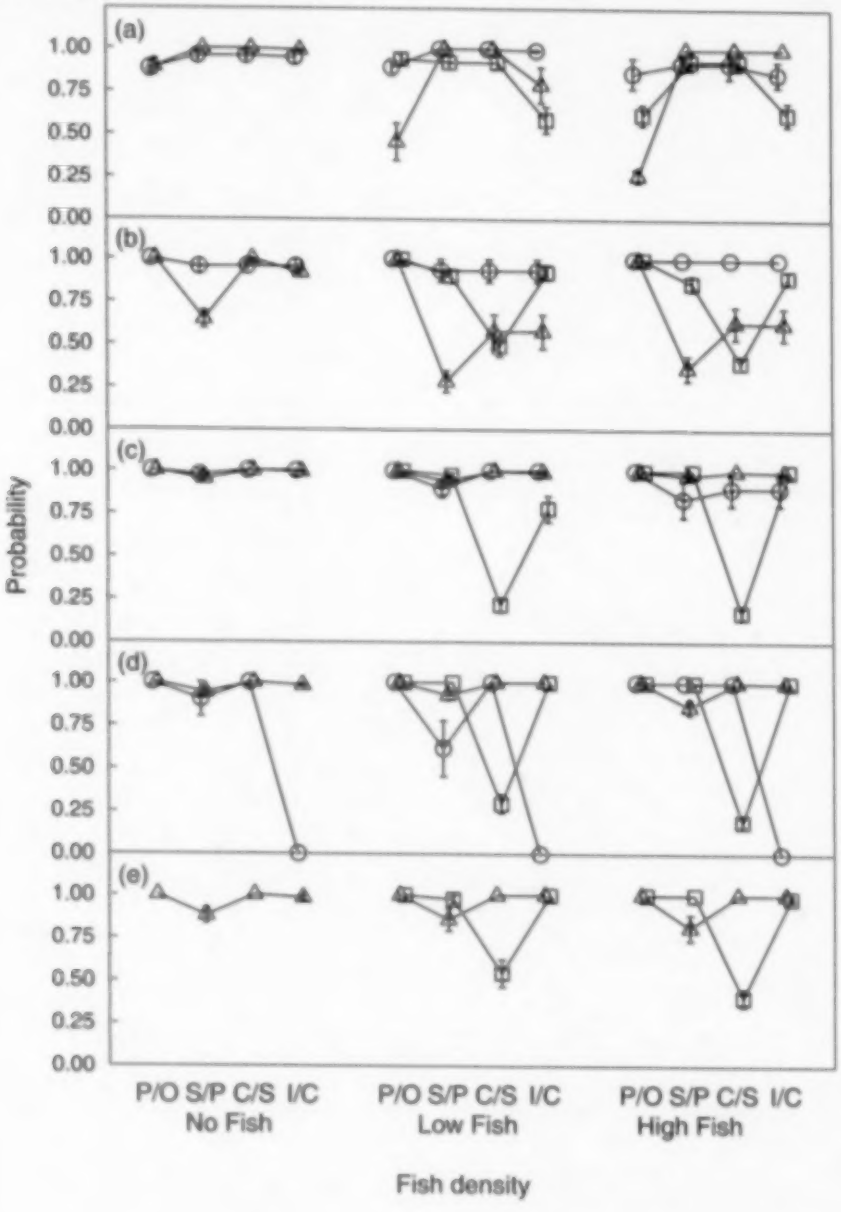
Fig. 3. Probability of pursuits per orientation (P/O), strikes per pursuit (S/P), captures per strike (C/S), and ingestions per capture (I/C) by (*a*) 20, (*b*) 40, (*c*) 60, (*d*) 80, and (*e*) 100 mm walleye (*Sander vitreus*) fed zooplankton, benthic invertebrates, and fish simultaneously. Behavioral probabilities were calculated for zooplankton (circles), benthic invertebrates (triangles), and fish (squares) at each fish density. Values are means ± 1 standard error.



consumed zooplankton. The number of benthic invertebrates eaten increased with benthos density but was lower when fish were present (Tables 1*c* and 1*d*). Fish consumption increased slightly with fish density (Table 1*c* and 1*d*) but was not affected by the presence of alternate prey.

The largest walleye (100 mm and 150 mm) were different from other sizes as they did not eat zooplankton (Fig. 1). For 100 mm fish, the few chironomids consumed increased with benthos density (Table 1*e*). Fish consumption was influenced by fish density and an interaction between zooplankton and benthos densities (Table 1*e*). In contrast, the 150 mm walleye did not consume benthic invertebrates in any of the prey combinations, and fish density did not significantly affect the number of fish

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Table 2. Results of the repeated-measures analysis of variance showing the between- and within-subject effects of walleye (*Sander vitreus*) length and zooplankton (*Z*), benthic invertebrate (*B*), and fish (*F*) densities on consumption of the three prey types during the 30 min experimental period.

		Zooplankton			Benthic invertebrates			Fish		
Source of variation	df	SS	*F*	*P*	SS	*F*	*P*	SS	*F*	*P*
Between subject										
Length	5	59.93	41.46	0.0001	61.31	69.37	0.0001	1.61	5.87	0.0001
Z	1	9.34	32.30	0.0001	0.38	2.16	0.14	0.54	9.94	0.002
B	1	4.23	14.64	0.0001	10.80	61.07	0.0001	1.48	27.11	0.0001
F	2	1.69	2.93	0.05	4.89	13.84	0.0001	15.95	145.60	0.0001
Error	466	134.73			82.37			25.52		
Within subject										
Time	5	27.64	40.33	0.0001	24.83	26.62	0.0001	3.25	9.88	0.0001
Time × length	25	23.53	6.87	0.0001	26.44	5.67	0.0001	3.26	1.98	0.003
Time × *Z*	5	11.91	17.38	0.0001	0.03	0.03	0.99	0.17	0.52	0.76
Time × *B*	5	0.55	0.80	0.55	3.89	4.17	0.0009	0.22	0.68	0.64
Time × *F*	10	1.07	0.78	0.65	2.12	1.14	0.33	2.84	4.31	0.0001
Error	2330	319.40			434.67			153.55		

Note: Experiments were divided into six 5 min intervals to examine effects of time on consumption. Walleye (20–150 mm) were fed the three prey types simultaneously in pool experiments. Sum of squares (SS) are type III (SAS Institute Inc. 2001).

consumed ($F_{[2,24]} = 2.08$, $P = 0.15$). The number of fish consumed was similar between 100 and 150 mm walleye ($F_{[7,35]} = 0.74$, $P = 0.64$).

Prey selectivity

Manly–Chesson's α values indicate that fish were selected when available for all sizes of walleye (Fig. 2). Selection of zooplankton and benthic invertebrates changed as walleye increased in size. The 20 mm walleye neither selected nor avoided zooplankton and benthic invertebrates when either no fish or low densities of fish were present (Fig. 2). At high fish densities, zooplankton (with the exception of the high zooplankton – low benthos treatment) and benthic invertebrates were selected against, but fish were selected at both low and high fish densities (Fig. 2). Benthic invertebrates were not selected by these small sizes of walleye in all treatment combinations (Fig. 2). At 40 mm, chironomids were selected when fish were not available, whereas only fish were selected in treatments when present (Fig. 2). Similar patterns were observed for the 60 mm walleye, although benthic invertebrates, not fish, were selected in the high zooplankton – low benthos density trials, even when fish were present (Fig. 2). Larger sizes of walleye (80 mm and 100 mm) selected fish whenever present and benthic invertebrates when fish were not present (Fig. 2). Selectivity indices were not calculated for the 150 mm size class because the walleye only consumed fish.

Foraging behaviors

Foraging efficiency varied with prey type, walleye length, and fish density (Fig. 3). Pursuits per orientation differed with prey type ($F_{[2,684]} = 55.78$, $P = 0.0001$), length ($F_{[4,684]} = 139.29$, $P = 0.0001$), the interaction between prey types and length ($F_{[7,684]} = 53.51$, $P = 0.0001$), and fish density ($F_{[2,684]} = 6.43$, $P = 0.0002$). Pursuit efficiencies varied with prey type for the 20 mm walleye (zooplankton > fish > benthic invertebrates) but were similar within a size class for walleye 40 mm and larger. The most noticeable differences in pursuit efficiency occurred for the 20 mm size class in which the probability of pursuing a chironomid upon orientation was less than 50% when fish were present (Fig. 3). Pursuit efficiency was near 100% for other size classes and prey types. Strikes per pursuit differed significantly with prey type ($F_{2,684} = 63.58$, $P = 0.0001$), walleye length ($F_{[4,684]} = 47.13$, $P = 0.0001$), and the interaction between prey type and length ($F_{7,684} = 24.45$, $P = 0.001$). The probability that a walleye would strike at chironomids decreased with walleye size (Fig. 3). Small walleye (20 and 40 mm) had greater strike efficiencies on zooplankton, whereas larger walleye had higher strike efficiencies on fish.

Capture efficiency (captures per strike) differed with prey type ($F_{[2,684]} = 36.20$, $P = 0.0001$), walleye length ($F_{[4,684]} = 23.94$, $P = 0.0001$), the interaction between prey type and length ($F_{[7,684]} = 23.81$, $P = 0.001$), and fish density ($F_{[2,684]} = 8.35$, $P = 0.0003$). Probability of successfully capturing different prey types was similar among the 20 mm walleye (Fig. 3). For 40 mm walleye, capture efficiency was lowest for fish and decreased for benthic invertebrates when fish were present (Fig. 3). Walleye 60 mm and larger had high capture efficiencies on zooplankton and benthos, but capture efficiencies on fish were less than 50% (Fig. 3). Ingestion efficiency (ingestions per capture) varied with prey type ($F_{[2,684]} = 32.60$, $P = 0.0001$), walleye length ($F_{[4,684]} = 23.94$, $P = 0.0001$), and the interaction between prey type and length ($F_{[7,684]} = 23.81$, $P = 0.001$). The probability that a walleye would retain a captured daphnid was high for walleye measuring 20–60 mm but almost 0% for 80 mm walleye (Fig. 3). As walleye increased in size, the probability that a walleye would retain a captured chironomid or fish increased (Fig. 3).

Captures of each prey type decreased over the 30 min trial as a function of each respective prey density (Table 2; Fig. 4); fewer captures occurred at lower densities. In addition, there was an interaction between time and walleye

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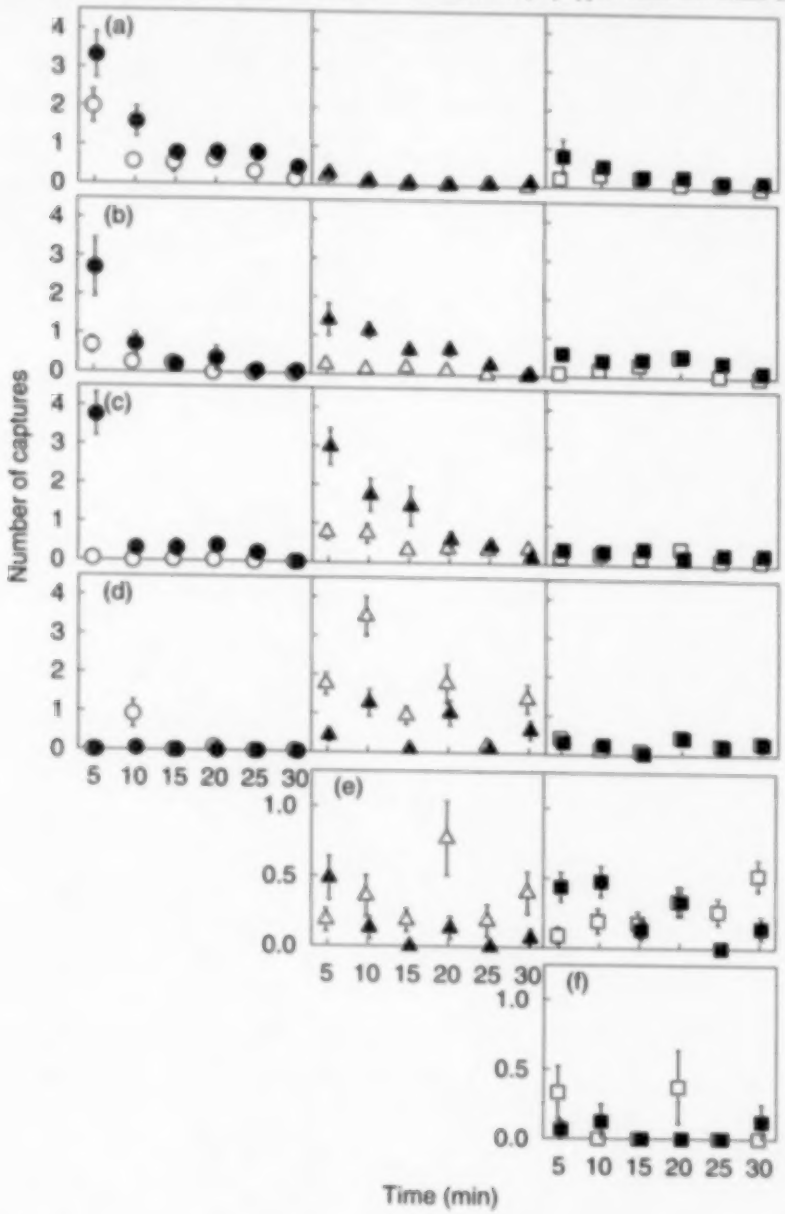
Fig. 4. Number of zooplankton (circles), benthic invertebrates (triangles), and fish (squares) captured during 5 min intervals of the 30 min experimental feeding period by (*a*) 20, (*b*) 40, (*c*) 60, (*d*) 80, (*e*) 100, and (*f*) 150 mm walleye (*Sander vitreus*). Densities of each prey type (low, open symbols; high, solid symbols) influenced capture of each respective prey type. Values are means ± 1 standard error.



length (Table 2): as walleye increased in size, differences over time were less noticeable. The timing of the initial captures of zooplankton, benthic invertebrates, and fish varied with prey type ($F_{[2,708]} = 43.29$, $P = 0.0001$), walleye length ($F_{[5,708]} = 4.26$, $P = 0.0008$), and the interaction between prey type and length ($F_{[7,708]} = 2.88$, $P = 0.006$). Walleye generally captured zooplankton earlier than chironomids and fish, and small (20 and 40 mm) walleye were quicker to capture prey than larger (100 and 150 mm) walleye (Fig. 5).

Discussion

YOY piscivores undergo ontogenetic changes in consumption and selection of prey. Walleye across a range of sizes can readily eat zooplankton, benthic invertebrates, and fish

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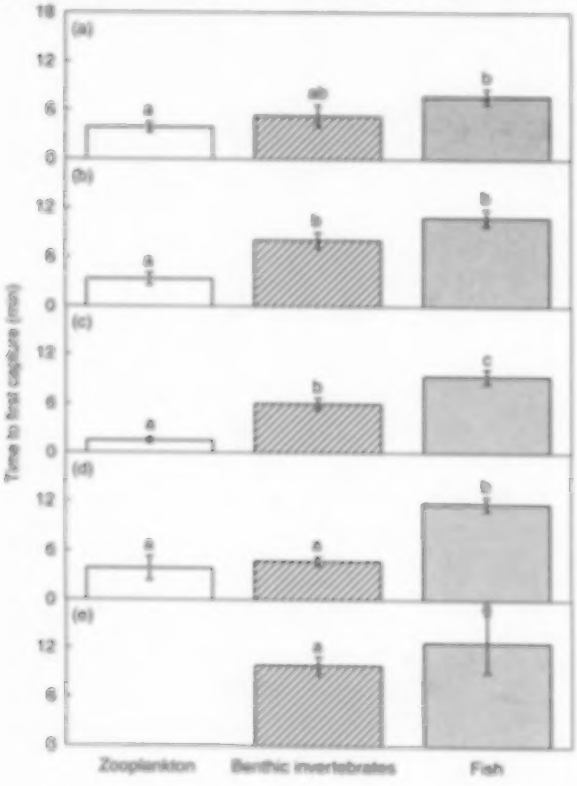
Fig. 5. Time (min) of initial capture of zooplankton (open bars), benthic invertebrates (hatched bars), and fish (shaded bars) by (*a*) 20, (*b*) 40, (*c*) 60, (*d*) 80, and (*e*) 100 mm walleye (*Sander vitreus*) during feeding experiments. Letters indicate statistical groupings within a size class. Values are means ± 1 standard error.



when only one prey type is present (Galarowicz and Wahl 2005), but a different pattern emerges when all three prey types are available. In our experiments, fish were almost always selected regardless of walleye size. Zooplankton were selected by early juveniles (20 mm) in some treatments but not by larger walleye (>40 mm). Benthic invertebrates were selected by 40–100 mm walleye when fish were not present, with consumption peaking with the 60 and 80 mm fish. Relative prey densities altered ontogenetic patterns of foraging with availability of more beneficial prey types determining consumption.

The presence and densities of the most energetically profitable prey shaped the observed foraging patterns. Prey fish are always more profitable (energy return ($J \cdot s^{-1}$)) than either zooplankton or benthic invertebrates for juvenile walleye (Galarowicz and Wahl 2005). Daphnids and chironomids have similar energy returns for the smallest walleye size class (20 mm), whereas energy return for benthic invertebrates is greater than zooplankton for larger (40–150 mm) walleye. We found that consumption of relatively less profitable prey types was in part influenced by whether or not more profitable prey were available. For example, consumption of zooplankton was determined not only by the number of daphnids available, but also by the density of chironomids and fish. Benthic invertebrate density and the density of the more profitable fish influenced the number of chironomids eaten. However, only the density of fish, not the densities of the invertebrate groups, influenced the number of fish consumed. In contrast, the ontogenetic shift from zooplankton to benthic invertebrates for yellow perch (*Perca flavescens*) has been attributed to a decline in zooplankton abundance rather than the presence of chironomid larvae (Wu and Culver 1992). Similarly, a shift from zooplanktivory to piscivory in age-0 Eurasian perch and zander (*Stizostedion lucioperca*) in the field was explained by declines in zooplankton density (Mehner et al. 1996). Our results suggest that ontogenetic shifts in other species should be examined in the context of prey profitability, relative prey availability, and predator foraging abilities rather than prey density alone.

Ontogenetic changes in consumption can be partly explained by foraging efficiencies. Although many studies have examined foraging efficiencies by different size predators on one or similar prey types (Mittelbach 1981; Juanes 1994; Lundvall et al. 1999), comparisons among drastically different prey types have not been made previously. Small juveniles were less likely to pursue chironomids than zooplankton and fish and less successful at retaining captured fish (20 mm) or were less likely to successfully prey upon chironomids when fish were available (40 mm). For walleye greater than 40 mm, the probability of successfully capturing a fish after striking at it was low compared with the invertebrate taxa, most likely because of the fish's predator-avoidance capabilities. Although fish were also not always eaten first, juvenile walleye are less likely to consume zooplankton or benthic invertebrates after capturing the energetically more valuable fish prey. In contrast, other invertebrate and vertebrate aquatic predators include more of less profitable prey types as a foraging bout progresses (Confer and O'Bryan 1989; Hirvonen and Ranta 1996). However, disparities among prey profitability in these studies were likely much smaller given that prey taxa were strictly plankton. These results suggest that foraging decisions in juvenile fishes can be influenced by the range of prey profitabilities available.

The ontogenetic shifts in prey selection and foraging behaviors we observed are reflected in growth. We reanalyzed data from Galarowicz and Wahl (2005), who examined absolute growth of YOY walleye of different sizes fed each prey type. Relative growth ($g \cdot g^{-1} \cdot day^{-1}$) did not differ among small walleye (20 and 40 mm) fed zooplankton and benthic invertebrates but is higher on fish (ANOVA: 20 mm, $F_{[2,14]} = 25.04$, $P = 0.0001$; 40 mm, $F_{[2,18]} = 5.80$, $P = 0.01$; Fig. 6). Walleye larger than 40 mm actually lose weight when feeding exclusively on plankton, exhibit moderate growth on benthic invertebrates, and have the highest growth when fed fish (ANOVA: 60 mm, $F_{[2,14]} = 66.63$, $P = 0.0001$; 80 mm, $F_{[2,12]} = 154.95$, $P = 0.0001$; 100 mm, $F_{[2,12]} = 5.15$, $P = 0.03$; Fig. 6). These same patterns are reflected in prey selection by similar sizes of walleye. Walleye always selected fish, which provides the greatest growth benefit. In addition, we saw a change in selectivity from zooplankton to benthic invertebrates with size that corresponded with these changes in growth. Changes in growth rate have been associated with single switches among prey types for a variety of fishes, including pumpkinseed sunfish (*Lepomis gibbosus*) (Osenberg

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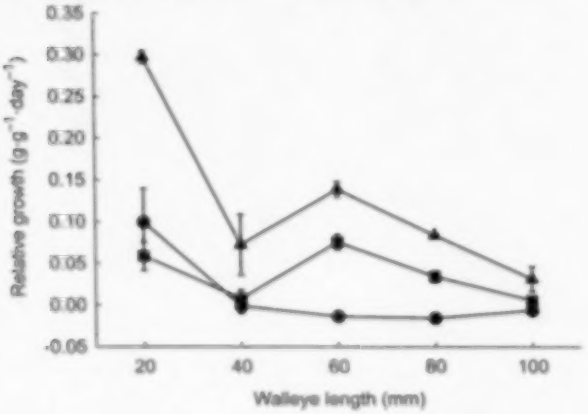
Fig. 6. Relative growth ($g \cdot g^{-1} \cdot day^{-1}$) of walleye (*Sander vitreus*) of different sizes (20, 40, 60, 80, and 100 mm) fed either zooplankton (circles), benthic invertebrates (squares), and fish (triangles) ad libitum. Values are means ± 1 standard error. Methods for these growth experiments are found in Galarowicz and Wahl (2005). Growth data ($g \cdot day^{-1}$) were reanalyzed as a function of initial walleye weight (Galarowicz and Wahl 2005).



et al. 1992), largemouth bass (*Micropterus salmoides*) (Adams and DeAngelis 1987; Wicker and Johnson 1987; Olson 1996), bluefish (*Pomatomus saltatrix*) (Buckel et al. 1998), and pikeperch (*Stizostedion lucioperca*) (Buijse and Houthuijzen 1992). Increased growth in the field has also been documented for percids switching from zooplanktivory to benthivory (Fox 1989) or zooplanktivory–benthivory to piscivory (Stahl and Stein 1994). Understanding the complex relationships among predator size, prey availability, foraging behaviors, and energy return can ultimately aid in predicting the variable growth and survival of fishes across a range of environmental conditions.

Besides changes in foraging abilities and prey profitability, factors such as habitat selection and predation risk likely shape diets in the field. The feeding patterns that we found in the laboratory occur concomitantly with shifts in habitat use. Larval walleye are found in pelagic areas and move inshore at approximately 30 mm (Houde and Forney 1970; Bulkey et al. 1976). This habitat shift has been attributed to an increase in inshore benthic invertebrates and fish availability (Fox 1989; Pratt and Fox 2001) and increased offshore predation risks (Pratt and Fox 2001). Yellow perch and bluegill undergo offshore-to-inshore shifts attributed to prey availability and predation risk trade-offs (Kelso and Ward 1977; Whiteside et al. 1985; Werner and Hall 1988). Because piscivores typically hatch earlier than other fish, they can exploit high zooplankton densities and then switch to macroinvertebrates or fish when the more valuable prey become available (Adams and DeAngelis 1987; Phillips et al. 1995; Gopalan et al. 1998).

Our data suggest that foraging patterns of juvenile piscivores are the result of a combination of ontogenetic changes in predator ability and relative prey profitability. Prey selection patterns are explained by predator ability to capture and handle different prey types, which ultimately determines growth. Juvenile piscivores maximize energy return given prey profitability and foraging efficiency, and as a result, fish are always selected when available, resulting in decreased consumption of less profitable prey types. Examining ontogenetic changes in foraging and resultant growth between closely related piscivores and nonpiscivores (see also Mittelbach and Persson 1998) would expand our understanding of evolutionary factors influencing ontogenetic diet shifts. We have linked ontogenetic foraging patterns of a juvenile piscivore with prey densities and profitability, but similar research on other species to assess generality is warranted.

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Short-term responses to watershed logging on biomass mercury and methylmercury accumulation by periphyton in boreal lakes

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Abstract: In the boreal forest, watershed logging may increase runoff, as well as chemical loading, including nutrient, dissolved organic carbon, and mercury, to lakes. Because they are exposed directly to nutrients and contaminants exported from the watershed, littoral communities such as periphyton may respond quickly to watershed disturbances. The objectives of this study were to evaluate the response of periphyton to watershed logging using a BACI (before–after control–impact) statistical approach and to develop a predictive tool to facilitate the elaboration of practical logging policies aimed at reducing Hg loading to lakes. In this study, we compare the periphyton biomass in 18 boreal Canadian Shield lakes, as well as their total mercury and methylmercury levels. During the ice-free season from 2000 to 2002, eight of these lakes were monitored before and after logging, with the other 10 lakes serving as controls. The BACI statistical analyses reveal a significant impact of logging on periphyton biomass (decrease; 0.6- to 1.5-fold) and methylmercury accumulation (increase; 2- to 9.6-fold). This study demonstrates that periphyton responds quickly to disturbances of the watershed. Our results suggest that the periphyton and watershed characteristics could serve as good management tools and that logging should be limited in watersheds with a mean slope below 7.0%.

Résumé : En forêt boréale, la coupe forestière peut augmenter l'écoulement et l'apport d'éléments chimiques aux lacs tel que le carbone organique dissous et le mercure. Les communautés littorales, comme le périphyton, peuvent être exposées directement aux apports de contaminants exportés du bassin versant, et ainsi répondre rapidement aux perturbations de ce dernier. Les objectifs de cette étude étaient d'évaluer la réponse du biofilm périphytique aux coupes forestières en utilisant une approche statistique de type BACI, et de développer des outils prédictifs utiles pour l'élaboration de politiques forestières visant la réduction des apports de Hg dans les lacs. Dans cette étude, nous avons échantillonné 18 lacs boréaux du bouclier canadien et comparé la biomasse et les concentrations de mercure total et méthylmercure du périphyton. Durant la saison libre de glace de 2000 à 2002, huit lacs ont été échantillonnés avant et après la coupe alors que les autres ont servi de témoins. Les analyses statistiques BACI révèlent un impact significatif des coupes sur la biomasse (diminution; 0,6 à 1,5 fois) et sur les concentrations de méthylmercure (augmentation; 2 à 9,6 fois). Cette étude démontre que le périphyton répond rapidement à une perturbation du bassin versant. Nos résultats suggèrent que le périphyton et les caractéristiques du bassin versant peuvent être de bons outils de gestion et que la coupe forestière devrait être limitée lorsque la pente moyenne est inférieure à 7,0 %.

Introduction

In Canada, boreal forests account for more than 50% of the territory (Burton et al. 2003), and in the Province of Quebec, freshwater environments occupy approximately 10% of the territory, with in excess of 500 000 lakes (Natural Resources Canada 1999). Timber harvesting in this region has increased during the last two decades and concerns have been raised over its potential impact on aquatic ecosystems (Burton et al. 2003).

Watershed disturbances, such as logging, increase chemical loading to lakes, including nutrient and dissolved organic carbon (DOC) (Rask et al. 1998; Carignan et al. 2000; Lamontagne et al. 2000). In turn, logging may also impact the benthic (Rask et al. 1998; Planas et al. 2000) and pelagic algal productivity (Rask et al. 1998; Planas et al. 2000; Steedman 2000).

In the boreal Canadian Shield forest, the soil is podsolic and rich in organic matter (Clayton et al. 1978) and, hence, readily adsorbs and accumulates Hg from dry and wet atmo-

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spheric deposition (St. Louis et al. 1996). In turn, boreal Canadian Shield forest watersheds serve as large reservoirs of Hg that shed their metal load when the soil and the land hydrology are disrupted by timber harvesting. Given the strong affinity of mercury (Hg) for DOC (Meili et al. 1991; Mierle and Ingram 1991; Kolka et al. 2001) and the well-documented increase in DOC loading to aquatic ecosystems upon logging (Lamontagne et al. 2000), a concurrent increase in Hg levels in aquatic organisms is typically observed in lakes after logging (Rask et al. 1994; Garcia and Carignan 1999, 2005).

Before the 1990s, most studies on the impact of logging focused on surface runoff waters (e.g., Likens et al. 1970). Investigations of lake nutrient loading from the watershed and lake communities in the pelagic zone are mostly recent (e.g., Lamontagne et al. 2000; Planas et al. 2000; Steedman 2000). Futhermore, few studies have focussed on the response to logging of littoral communities in lakes (Rask et al. 1998; Planas et al. 2000), and the impact of metal loading in the littoral zone of boreal forest lakes has most often been ignored. In lakes, the littoral zone may encompass a significant proportion of the primary production of the whole lake system of which the attached community, the periphyton, can account for a greater fraction of the primary production than the phytoplankton (Wetzel 1964; Planas et al. 2000). Periphyton is located at the interface between watersheds and lakes; it can retain and recycle chemical elements from runoff water (Borchardt 1996) and thus can rapidly respond to watershed disturbances (Rask et al. 1998; Planas et al. 2000) and serve as an early and powerful biomonitor of these disturbances. Likewise, littoral communities will respond quickly to Hg loading after watershed logging. In addition, the periphyton communities contribute to the methylation of inorganic Hg (Guimarães et al. 1998; Cleckner et al. 1999; Desrosiers et al. 2006*a*), and given that they are grazed upon by macroinvertebrates, periphyton may represent a significant source of Hg to fish (Rask et al. 1994; Hill et al. 1996; Cleckner et al. 1998).

In a previous study on the impacts of logging on lake communities, algal biomass in periphyton was found to be higher in perturbed lakes (i.e., after logging and wildfire) than in control lakes (Planas et al. 2000). In the absence of before-disturbance data, the increase in periphyton biomass and methylmercury accumulation observed in perturbed lakes could not be unambiguously ascribed to the disturbance but may have reflected interlake variations. In view of this potential shortcoming, we carried out a study on before and after disturbance to evaluate the impact of logging on (*i*) periphyton algal biomass and dry mass (Chl *a*, DM) and (*ii*) total mercury (THg) and methylmercury (MeHg) accumulation in the littoral zone of the boreal lakes. To distinguish the impact of the disturbance from other variables, we applied the BACI (before–after control–impact) sample design that requires data from control and target sites sampled before and after disturbance.

Materials and methods

Study site and sampling

The study was carried out on 18 boreal lakes located in the Grenville geological province of the Canadian Shield and distributed over a large area (47°07′ to 50°07′N, 73°06′ to 76°43′W; Fig. 1). The soils of the area cover glacial till deposits that are generally thin (i.e., 0–2 m). The study area is located at the transition zone between the boreal mixed and coniferous forest and is mainly composed of black spruce (*Picea mariana*), jack pine (*Pinus divaricata*), white birch (*Betula papyrifera*), and trembling aspen (*Populus tremuloides*). Over the period of this study (i.e., 2000–2002), eight lakes had their watersheds logged over 15%–79% of their area. The other 10 lakes are used as control sites to document interannual variations.

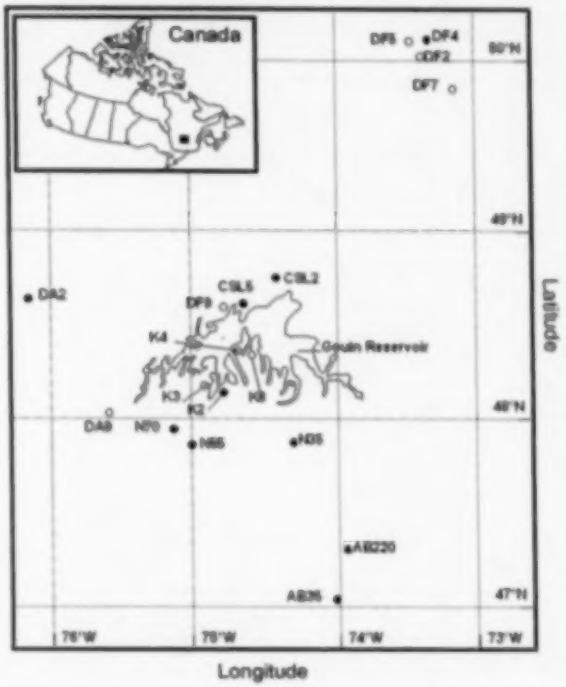

Fig. 1. Location of the 18 study lakes in the Eastern Canadian Shield around the Gouin reservoir (Province of Quebec, Canada): control lakes (●); experimental, logged lakes (○). Location in Canada is illustrated in the inset.

This project is a component of the Sustainable Forest Management Network (SFM Network) research program and benefited from the water quality and physiographic data acquired by other research groups within the program. The variables and their respective methods of acquisition are identical to those described in former studies (D'Arcy and Carignan 1997; Carignan et al. 2000; Lamontagne et al. 2000). The watershed delineation and wetlands area measurements were derived from provincial topographic maps (1:20 000); the mean watershed slope was measured after digitizing the topography of each drainage area to generate 10 m resolution digital elevation models (ArcInfo; ESRI 7, Redlands, Calif.; D'Arcy and Carignan 1997; Carignan et al. 2000). Lake morphometry was established from bathymetric maps produced from aerial photographs and 15–25 echosounder transects (Carignan et al. 2000).

Sampling

Fourteen study lakes were sampled once a year during two consecutive ice-free seasons, before and after logging, and

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another four lakes were sampled 2 years after logging (DA9, K3, K4, and K8). Harvesting was carried during the winter, and consequently, we sampled the periphyton a few months (year-1) and more than a full year (year-2) after logging. The lakes were sampled between the end of June and mid-August. A statistical analysis of periphyton biomass and mercury concentration data within natural lakes and lakes before logging as functions of temperature and sampling date (i.e., Julian day) revealed no significant trend in the same lakes (linear regression, $p > 0.1$; Desrosiers et al. 2006*b*). Our inability to distinguish the impact of temperature on these variables is probably related to the small range (18–22 °C) of water temperatures. To assess the impact of logging, samples were recovered on successive years within 18 ± 16 Julian days of the first year's sampling date. During the second sampling year, three lakes (AB35, CSL2, CSL5) were sampled more than 35 Julian days after the original sampling date, and samples were recovered from the other 15 lakes within 11 ± 6 Julian days. The lakes offer a similar substrate (i.e., rocky shore) for periphyton colonization and growth and support similar diatom-dominated algal communities (Z. Beyruth, Agência Paulista de Agronegócios SAASP, Instituto de Pesca, Av. Plinio de França n. 85 Ilha Anchieta, Saco da Ribeira, Ubatuba CEP: 11680-000, São Paulo, Brazil, personal communication). Periphyton were quantitatively collected from their rocky substrates with a modified brush-type sampler (Vis et al. 1998; Desrosiers et al. 2006*b*) at two sites on opposite shores of the lakes. Three replicate samples were collected randomly at a depth of 0.5 m over a 2 m^2 area at each site and pumped into 3 L dark polycarbonate bottles. Three subsamples (100 mL) of the thoroughly stirred periphyton slurry were filtered through untreated Whatman GF/C filters for chlorophyll *a* (Chl *a*) analysis, as well as through precombusted (500 °C for 90 min) and preweighed filters for the dry mass (DM) determinations. Six more subsamples were filtered through precombusted Whatman GF/C filters for each of the THg and MeHg analyses.

Determination of periphyton characteristics

Chl *a* was extracted using a hot 90% ethanol solution, and its absorbance was measured spectrophotometrically before and after acidification (Sartory and Grobbelaar 1984). Dry mass (DM) was measured after oven drying the filters at 40 °C until constant mass. Chl *a*, a measure of algal biomass, and DM are used to account for both the organic and inorganic matter content of the periphyton mats. The detrital, particulate inorganic and the organic matter content of the mats may increase in response to erosion and particle export from the watersheds after logging.

Methylmercury (MeHg) concentrations were determined following an aqueous-phase ethylation, Tenax® trap collection, isothermal gas chromatography, thermal decomposition, and cold-vapor atomic fluorescence detection (CVFAS; Bloom 1989; Gagnon et al. 1996). MeHg was extracted from six pooled freeze-dried filters with a solution of 25% KOH in methanol in screw-cap Teflon bottles mounted on a wrist-shaker overnight at room temperature. Replicate analyses of aliquots of each extract were carried out until a reproducibility of better than 10% was achieved, and the results were averaged. A certified reference material (DORM-2; National Research Council of Canada (NRC)) was analysed after every five samples. The average MeHg concentration of the reference material, determined by our protocol, was 4.39 ± 0.40 mg $Hg \cdot kg^{-1}$ ($n = 51$), corresponding to ~98% of the certified value (4.47 ± 0.32 mg $Hg \cdot kg^{-1}$) and well within the reported uncertainty. The instrumental detection limit was 10 pg and the analytical blanks were always under the detection limit.

In 2000 and 2001, THg concentrations were determined following acid digestion, gold trap preconcentration, thermal desorption, and CVFAS (Bloom and Fitzgerald 1988). Briefly, three freeze-dried filters were digested individually with a mixture of concentrated nitric and hydrochloric acid (10:1; Desrosiers et al. 2006*b*), aliquots of the digestates were analysed until the reproducibility was better than 10%, and results were averaged. A certified reference material (TORT-2; NRC) was analysed after every 10th sample. The average THg concentration of this material, determined by our protocol, was 0.292 ± 0.011 mg $\cdot kg^{-1}$ ($n = 51$) or ~108% of the certified value (0.270 ± 0.060 mg $\cdot kg^{-1}$) and well within the reported uncertainty. The instrumental detection limit was 5 pg, the analytical blanks were 18.0 ± 2.4 pg of Hg, and thus, the analytical detection limit was 26.4 pg of Hg.

In 2002, THg concentrations were determined following thermal decomposition, gold amalgamation, and atomic absorption spectrometry (combustion AAS) with a Direct Mercury Analyzer (DMA-80; Milestone, Inc., Monroe, Connecticut; Cizdziel et al. 2002). This protocol does not require pretreatment of the filters after they are freeze-dried. A certified reference material (TORT-2; NRC) was analysed after every 20 samples. The average THg concentration of the reference material, determined by the thermal decomposition protocol, was 0.295 ± 0.013 mg $\cdot kg^{-1}$ ($n = 22$) or ~109% of the certified value (0.270 ± 0.060 mg $\cdot kg^{-1}$) and well within the reported uncertainty of the standard. The instrumental detection limit was 10 pg and the analytical blanks were well below this detection limit. We cross-calibrated the two analytical methods (i.e., acid digestion and thermal decomposition) and found a very good correlation between them ($r^2 = 0.96$, $p < 0.0001$, slope 1:1, $n = 12$; data not shown), as did Cizdziel et al. (2002) when comparing the performance of both methods with fish tissue ($r^2 = 0.95$). Results from two lakes (i.e., N55 and DA9) were discarded because of obvious contamination problems.

Statistical approach

The detection of anthropogenic disturbance on natural systems is a critical stage in the process of management of natural resources and in the evaluation of anthropogenic impacts. Green (1979) proposed a statistical approach based on the comparison between one control and one impacted site, sampled before and after the disturbance. In our study, we chose an approach proposed by Underwood (1992, 1994) and this BACI design requires data from multiple control and target sites sampled before and after perturbation. Briefly, the statistical method is based on a series of one-way and two-way ANOVA (analysis of variance, general linear model (GLM)) to determine the difference before and after perturbation (*B*), as well as between impacted and control lakes (*I*)

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and their interaction (B-I). Finally, a Fisher test ($B_{impact}/B_{control}$) is used to assess if differences between before and after logging (B_{impact}) are larger than the interannual variability in control lakes ($B_{control}$). In turn, the statistical analyses conclusively indicate whether or not there is a significant impact of watershed logging on the variable of interest. Distribution normality and variance equality was verified and a $\log_{10}$ transformation was carried out to satisfy the ANOVA assumptions. If the distribution was not normal, significance was verified with a nonparametric test (Wilcoxon/Kruskall–Wallis; cluster 3; MeHg) and Welch ANOVA was used when variance was unequal (cluster 1; Chl *a* and DW).

The BACI statistical analysis calls for a comparison of similar control and perturbed sites to better assess the importance of the anthropogenic influence. Because of the wide variability in watershed and lake morphometric and physiographic characteristics in our study area (Table 1), we used a principal component analysis (PCA) to cluster lakes according to characteristics that can readily be used by forest managers and are known to influence the export of chemical elements from the watershed to the lakes and (or) the fate of these element in the lake (Carignan et al. 2000; Lamontagne et al. 2000). These variables include the drainage ratio (DR), wetland to lake area ratio (WA/LA), mean lake depth (Z_{mean}; m), mean watershed slope (SLO; %), and runoff (mm) (Table 1). The statistical analysis was performed using CANOCO (ter Braak and Smilauer 1998). The clustering determination and the difference in the position of the cluster centroids were tested with multiple discriminant analysis and Wilks' lambda test using JMP IN 5.1 (SAS Institute Inc. 2005). Clustering reduced the number of sites in each group to either a single logged lake or a control lake, greatly weakening the robustness of the BACI analysis. Under these conditions, the results should be interpreted with caution (Underwood 1994). Nevertheless, this approach remains very useful in identifying processes that warrant further investigations.

Linear statistical analyses were performed using JMP IN 5.1 (SAS institute Inc. 2005). Multiple regressions were carried out with stepwise forward–backward variable selection ($p < 0.05$ as an entry and keeping level). The homoscedasticity of the residual and the normality of the predicted–observed residuals were verified with a Shapiro–Wilk W test. The data were $\log_{10}$-transformed when necessary. The absence of correlations between variables used in stepwise multiple regression analyses was verified. One-way ANOVA was performed on the periphyton Chl *a* and MeHg concentrations in lakes sampled 2 years after logging. The normality and variance equality were verified and data were $\log_{10}$-transformed to satisfy the ANOVA assumptions. When ANOVA was significant, differences among groups were tested using Tukey's honestly significant difference (HSD) test for multiple comparisons.

Results

Clusters of lakes

In the PCA analysis (Fig. 2), axis 1 explained 34% of the variance, which was mainly correlated with wetland to lake area ratio (0.70) and runoff (–0.64) and partially with lake mean depth (–0.58). Axis 2 explained 23% of the variance (Fig. 2), which was positively correlated with mean watershed slope (0.76) and drainage ratio (0.74). Seventeen percent of the variance was explained by axis 3, which was mainly correlated with mean depth (0.74) and partially with runoff (0.54), both of which also defined axis 1. The PCA analysis allowed us to cluster the lakes into four distinct groups (Fig. 2), each cluster including at least one lake with a logged watershed and one control lake. The multiple discriminant analysis confirmed that lakes were well classified and that the positions of the cluster centroids were significantly different ($p = 0.0001$; Wilks' lambda test). The first cluster (DF5, DF7, and N55) was positively related with axis 2. These lakes are located in watersheds with high mean watershed slope (SLO) and drainage ratio (DR) (Fig. 2; Table 2). The second cluster of lakes (DF2, DF9, K3, K8, and CSL2) was negatively related with axis 2, their watershed characteristics contrasting with those of group 1 (i.e., low SLO and DR) (Fig. 2; Table 2). The third cluster (K4, DA2, K2, and DF4) was mainly related with axis 3, with these lakes characterised by intermediate mean depth and runoff (Fig. 2; Table 2). Finally, the fourth cluster (DA9 and CSL5) contained lakes with the highest drainage ratio in our study (Fig. 2; Table 2). Four control lakes (AB35, AB220, N35, and N70) were outliers, i.e., dissimilar to any perturbed lakes and thus were not included in the BACI analysis except when all lakes were considered (Fig. 2). The BACI analysis could not be applied to the THg concentrations of clusters 1 and 4 because THg data were not available in two lakes (N55 and DA9).

Table 1. Watershed physiographic and lake morphologic characteristics, as well as lake water physicochemical variables (range (mean)) of the 10 control and eight logged lakes before logging.

	Control	Logged
SLO (%)	4.5–18.1 (9.7)	3.0–10.5 (7.1)
LA (km^2)	0.2–1.4 (0.6)	0.2–0.8 (0.4)
DR	2.0–10.4 (4.8)	2.9–14.5 (6.5)
Z_{max} (m)	6.4–21.7 (12.6)	6.7–13.7 (9.3)
Z_{mean} (m)	2.6–7.8 (4.5)	2.3–3.4 (2.9)
Wetland (%)	0.2–26.8 (5.5)	0.0–11.0 (3.1)
WA/LA	0.0–0.9 (0.3)	0.0–0.5 (0.2)
Littoral (%)	16–61 (42)	7–61 (42)
Runoff (mm)	356–426 (401)	328–426 (385)
ϵ_{PAR} (m^{-1})	0.4–1.9 (1.3)	1.4–2.7 (1.9)
pH	5.9–6.5 (6.2)	5.9–6.8 (6.3)
DOC ($mg{\cdot}L^{-1}$)	4.1–9.4 (6.4)	6.2–12.7 (8.9)
TP ($\mu g{\cdot}L^{-1}$)	6.7–12.3 (8.8)	6.2–15.7 (10.8)
TN ($\mu g{\cdot}L^{-1}$)	175.9–289.4 (240.9)	218.7–395.3 (280.4)

Note: SLO, mean watershed slope (%); LA, lake area (km^2); DR, drainage ratio (drainage area/lake area); Z_{max}, maximum lake depth (m); Z_{mean}, mean lake depth (m); Wetland, percent of watershed covered by wetlands; WA/LA, wetland area/lake area; Littoral, littoral area/lake area × 100 (%); ϵ_{PAR}, light extinction coefficient (m^{-1}); DOC, dissolved organic carbon ($mg{\cdot}L^{-1}$); TP, total phosphorus ($\mu g{\cdot}L^{-1}$); TN, total nitrogen ($\mu g{\cdot}L^{-1}$). Data from R. Carignan (GRIL, Université de Montréal, Montréal, QC H3C 3J7, Canada, personal communication).

Logging impact on periphyton biomass

When all lakes before logging and control lakes were considered together, the range of periphyton biomass varied

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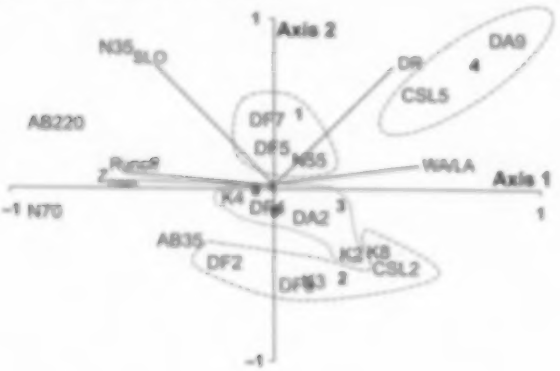
Fig. 2. Lake clusters classified according to a principal component analysis (PCA) as a function of watershed physiographic and lake morphologic variables. Axes 1 and 2 explain 34% and 23% of the variance, respectively.



widely: 5–240 mg·m^{-2} for Chl *a* (Table 2; Fig. 3*a*) and 12–690 g·m^{-2} for DM (Table 2). After logging, the median periphyton Chl *a* concentrations decreased in perturbed lakes (Fig. 3*a*) but the impact on DM was small (Fig. 3*b*), and no significant interannual variations were observed in control lakes (Figs. 3*a*, 3*b*). The periphyton Chl *a* responded strongly to logging, displaying a significant decrease in concentration in five of the eight logged lakes. Furthermore, in three of the four lakes sampled 2 years after logging, we observed a progressive decrease of the mean algal periphyton biomass (one-way ANOVA, $p < 0.01$; Fig. 4). The BACI statistical analyses also revealed a significant decrease of periphyton Chl *a* ($B_{impact}/B_{control}$) when all lakes were considered together (Table 3*a*) and for all lake clusters taken individually except cluster 3 (Table 3*a*). The BACI statistical design could not detect the impact of logging on periphyton DM as there were no significant differences between the logged and control lake interannual variability within any of the clusters ($B_{impact}/B_{control}$; Table 3*b*). Nevertheless, if we consider logged lakes individually, a significant decrease in DM after logging is noted in lakes in which we observed a significant effect on Chl *a*. The DM median also appeared to decrease after logging, but the difference was not significant (Fig. 3*b*).

In a predictive model, when all sampling years and lakes before and after logging were considered, 73% of the variance in periphyton Chl *a* was explained by the negative correlations to mean watershed slope and relative logging area (%log), as well as the positive correlations to latitude and wetland to lake area ratio (Table 4*a*). The same variables, minus latitude plus lake area, accounted for 64% of the periphyton DM variance (Table 4*b*). The percent area of the watershed logged explained 12% and 6% of the DM and Chl *a* variance, respectively (Tables 4*a*, 4*b*).

Logging impact on periphyton THg and MeHg concentrations

The range of prelogging Hg concentrations in periphyton was also very large, from 15 to 398 ng·g^{-1} (DM) for THg and from 1 to 68 ng Hg·g^{-1} (DM) for MeHg (Table 2). MeHg accounted for 1% to 36% of the THg. One or 2 years after logging, the median THg concentration in periphyton appeared to decrease (Fig. 5*a*), but the response was highly variable between lakes: THg concentrations in periphyton increased significantly in three (K3, K4, and K8) of the eight perturbed lakes, but only in the second sampling year following a decrease during the first year, whereas it significantly decreased in one lake (DF5) and showed no significant change in three lakes (DF2, DF7, DF9). Similarly, the interannual variability in control lakes was mixed: three lakes displayed a significant increase between 2000 and 2001 (CSL5, DF4, and N55), whereas no change was detected in the other seven lakes. Under these conditions, the BACI statistical analysis could not detect the impact of logging on periphyton THg concentrations in any of the lake clusters (Table 5*a*). A simple regression analysis, with the periphyton THg concentration as a dependent variable and the ratio of wetland to lake area as an independent variable, revealed a weak negative but, nonetheless, significant correlation ($r^2 = 0.26$, $p < 0.01$; Table 4*c*).

The periphyton MeHg concentrations increased within one year after logging in five of the eight perturbed lakes, whereas in the others, the increase was either statistically insignificant (DA9, K8; one-way ANOVA, $p > 0.1$) or undetectable (DF9). The increase of MeHg concentrations was progressive in the four logged lakes sampled two consecutive summers after the perturbation (Fig. 6). In some control lakes (AB35, DF4), we also observed interannual variability (mean of 3.5-fold increase) of MeHg levels in periphyton. The BACI statistical analysis suggests a significant impact of logging on MeHg concentrations in periphyton of lakes from cluster 2 (Table 5*b*), with a mean 2.5-fold increase in this cluster and up to a 9.6-fold increase in lake K3. In lake K3, the periphyton MeHg concentrations increased from 4.3 to 41.2 ng Hg·g^{-1} DM, even if only 26% of its watershed was logged. As noted previously, this cluster of lakes is characterised by low drainage ratio and watershed slope, as well as low MeHg concentrations before harvesting (i.e., twofold lower than the other lakes; *t* test, $p = 0.0010$; Table 2). The BACI statistical analysis did not detect a significant change of MeHg concentrations in periphyton after logging when all lakes were considered together or in the other three clusters. Its inability to detect an impact in the three other clusters was due to the high interannual variability in the control lakes (Fig. 5*b*; Table 5*b*). We observed a significant *F* ratio in cluster 4 ($B_{impact}/B_{control}$; Table 5*b*), but it does not seem to be related to logging because no significant difference was observed before and after perturbation in logged lakes ($B_{impact} = ns$; Table 5*b*). It most likely reflects a higher variability between sites within individual logging lakes.

The factor that best explains the variance in periphyton MeHg concentrations is bioconcentration, as revealed by a negative correlation with periphyton algal biomass (Chl *a*, $r^2_{partial} = 0.51$; Table 4*d*). The positive relationship with percent of watershed area logged (%log) is weak but nonetheless significant ($r^2_{partial} < 0.10$; Table 4*d*). When the periphyton biomass was removed as an independent variable in the stepwise statistical analysis, the MeHg concentrations were positively correlated with watershed slope, percentage of watershed logged, and runoff and negatively correlated with latitude ($r^2_{adjusted} = 0.49$, $p < 0.0001$; Table 4*d*).

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Table 2. Watershed, lake, and periphyton characteristics of control and before logging lakes (minimum – maximum) of all lakes and for the four clusters of lakes determined by principal component analysis.

	All lakes	Cluster 1	Cluster 2	Cluster 3	Cluster 4
%logged	0–79	0–79	0–72	0–44	0–15
SLO (%)	3.0–18.1	8.9–13	3.0–6.9	4.5–9.6	8.7–9.5
DR	2.0–14.5	4.2–8.6	2.3–6.4	4.5–5.5	10.4–14.5
Z_{mean} (m)	2.3–7.8	2.6–3.4	2.3–3.3	2.6–2.9	2.4–5.3
WA/LA	0.00–0.89	0.08–0.25	0.00–0.89	0.08–0.09	0.52–0.87
Runoff (mm)	306–594	387–419	318–399	318–387	349–376
Chl *a* ($mg \cdot m^{-2}$)	5–240	5–110	14–209	15–240	12–74
DM ($g \cdot m^{-2}$)	12–690	37–350	60–443	52–491	46–557
THg ($ng \cdot g^{-1}$ DM)	15–398	98–398	15–214	37–369	36–112
MeHg (ng $Hg \cdot g^{-1}$ DM)	1–68	1–30	1–14	1–23	3–17

Note: %logged, logging area/watershed area × 100 (%); SLO, mean watershed slope (%); DR, drainage ratio; Z_{mean}, mean lake depth (m); WA/LA, wetland area/lake area; Chl *a*, periphyton algal biomass ($mg \cdot m^{-2}$); DM, periphyton dry mass ($g \cdot m^{-2}$); THg and MeHg, periphyton mercury and methylmercury concentrations before logging. Morphological data from R. Carignan (GRIL, Université de Montréal, Montréal, QC H3C 3J7, Canada, personal communication).

Fig. 3. Box plots showing median values and minimum, 25th, 75th, and maximum percentiles of periphyton biomass: (*a*) algal chlorophyll *a* biomass (Chl *a*; $mg \cdot m^{-2}$), (*b*) total dry mass (DM; $g \cdot m^{-2}$) in control lakes (shaded bars; years 1 and 2) and experimental lakes (open bars; before and the first summer after logging).

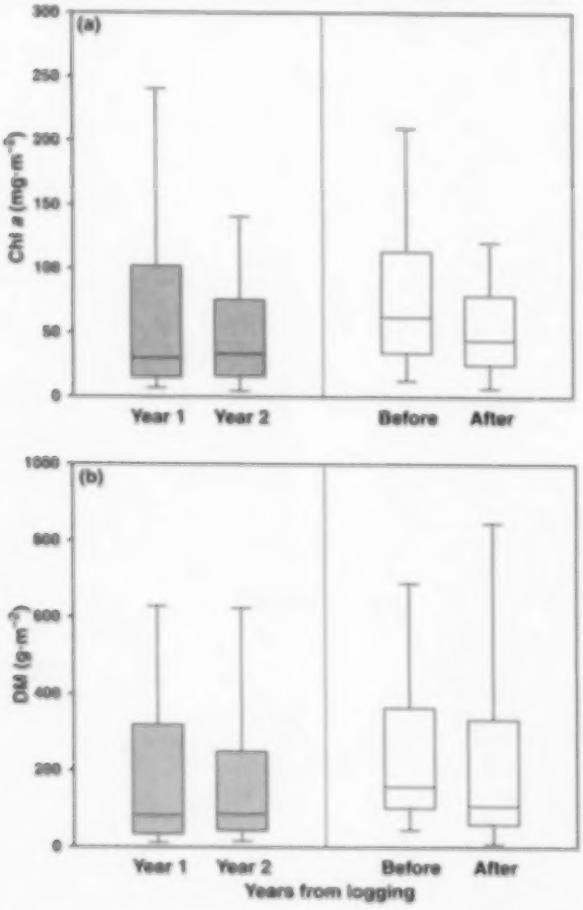
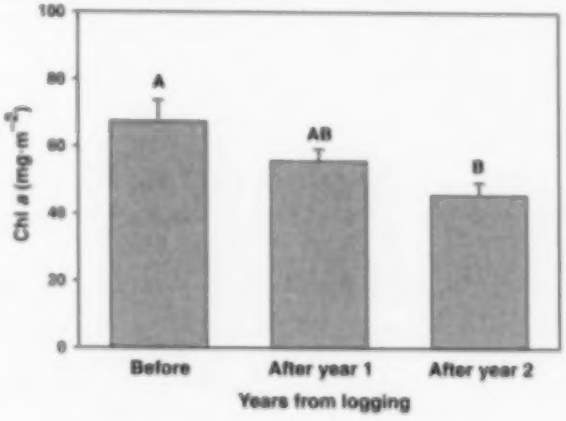


Fig. 4. Periphyton algal chlorophyll *a* biomass (Chl *a*; mean ± standard error) in experimental lakes sampled before and two consecutive summers after logging. Different letters indicate significant mean differences (one-way analysis of variance, $p < 0.01$) within sampling years.



Discussion

Impact of logging on periphyton biomass

The BACI statistical analyses reveal a significant impact of logging on Chl *a* in all lake clusters except cluster 3. This cluster is characterised by high periphyton biomass before logging (1.5-fold; *t* test, $p < 0.0001$) and less than 44% of the watershed area harvested. The higher mean per-unit-surface-area biomass (1.2-fold) translates into thicker periphyton mats in lakes of cluster 3 and may render this community more resilient to disturbances because the presence of a thick boundary layer favours the internal cycling of nutrients and carbon (Borchardt 1996) and makes algal growth less susceptible to external perturbations. In addition, there is a significant interannual variation among the control lakes of cluster 3. The combination of these factors prevents the detection of an unambiguous impact of logging in this clus-

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Table 3. The before–after control–impact (BACI) statistical analysis of logging on periphyton biomass: (*a*) chlorophyll *a* (Chl *a*; mg·m^{-2}); (*b*) dry mass (DM; g·m^{-2}).

	All lakes		Cluster 1		Cluster 2		Cluster 3		Cluster 4	
Source of variation	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*
(*a*) Chl *a*										
Before and after (*B*)	0.32	3.54 ns	0.31	4.03 ns	0.05	1.26 ns	0.61	11.78 *	0.21	7.75 *
Impact vs control (*I*)	2.00	21.98 *	1.05	13.52 *	0.21	4.77 *	0.46	8.42 *	0.14	5.09 *
B·*I*	0.10	1.05 ns	0.23	2.97 ns	0.04	0.99 ns	0.003	0.01 ns	0.20	7.40 *
$B_{control}$	0.001	0.30 ns	0.003	0.03 ns	0.0002	0.01 ns	0.29	4.56 *	0.0001	0.002 ns
B_{impact}	0.50	13.13 *	0.66	9.90 *	0.32	9.02 *	0.14	8.57 *	0.48	15.06 *
$B_{impact}/B_{control}$		500.00 *		200.00 *		1600.00 *		0.48 ns		4800.00 *
(*b*) DM										
Before and after (*B*)	0.81	6.25 *	0.62	8.30 *	0.32	4.46 *	0.88	27.66 *	0.06	0.47 ns
Impact vs control (*I*)	1.50	11.53 *	0.11	1.54 ns	1.34	18.85 *	0.01	3.34 ns	0.27	2.30 ns
B·*I*	0.32	2.44 ns	1.04	13.93 *	0.11	1.54 ns	0.01	0.24 ns	0.34	2.88 ns
$B_{control}$	0.05	1.55 ns	0.02	0.55 ns	0.02	0.58 ns	0.64	17.24 *	0.05	1.55 ns
B_{impact}	0.05	0.34 ns	1.74	37.55 *	0.58	8.65 *	0.15	6.87 *	0.39	2.30 ns
$B_{impact}/B_{control}$		1.00 ns		87.00 ns		33.80 ns		0.23 ns		7.80 ns

Note: MS, mean square error; *F*, Fisher test *F* ratio; an asterisk (*) indicates significant effect; ns indicates no significant effect.

Table 4. Stepwise multiple regression models carried out with all samples of (*a*) periphyton algal biomass (Chl *a*; mg·m^{-2}), (*b*) periphyton dry mass (DM; g·m^{-2}), (*c*) periphyton THg concentrations (ng Hg·g^{-1} DM), and (*d*) periphyton MeHg concentrations (ng Hg·g^{-1} DM).

(*a*) $\log_{10}$(Chl *a*) = –(8±2) + (0.21±0.03)lat – (0.04±0.01)SLO – (0.003±0.001)$\sqrt{\%}\log$ + (0.2±0.1)WA/LA

$r^2_{partial}$ 0.43 0.21 0.06 0.06

RMSE = 0.800 $r^2_{adjusted}$ = 0.73 $p < 0.0001$ $n = 41$

(*b*) $\log_{10}$(DM) = (3.1±0.2) – (0.08±0.01)SLO – (2.1±0.5)$\log_{10}$(LA) – (0.004±0.002)$\sqrt{\%}\log$ + (0.3±0.1)WA/LA

$r^2_{partial}$ 0.31 0.15 0.12 0.06

RMSE = 1.49 $r^2_{adjusted}$ = 0.64 $p < 0.0001$ $n = 41$

(*c*) $\log_{10}$(THg) = (2.2±0.1) – (0.5±0.1)WA/LA

RMSE = 0.22 r^2 = 0.26 $p < 0.01$ $n = 29$

(*d*) $\log_{10}$(MeHg) = (2.3±0.2) – (0.9±0.1)$\log_{10}$(Chl *a*) + (0.004±0.001)$\sqrt{\%}\log$

$r^2_{partial}$ 0.51 0.06

RMSE = 1.14 $r^2_{adjusted}$ = 0.55 $p < 0.0001$ $n = 41$

$\log_{10}$(MeHg) = (6±2) + (0.04±0.01)$\sqrt{\%}$SLO + (0.005±0.002)$\sqrt{\%}\log$ – (0.19±0.05)lat + (1.4±0.5)$\log_{10}$(runoff)

$r^2_{partial}$ 0.21 0.10 0.13 0.10

RMSE = 1.89 $r^2_{adjusted}$ = 0.54 $p < 0.0001$ $n = 41$

Note: RMSE, root mean square error; lat, latitude (°); SLO, mean watershed slope ($\sqrt{\%}$); $\sqrt{\%}\log$, proportion of watershed logged ($\sqrt{\%}$); WA/LA, wetland to lake area ratio; LA, lake area (km^2); p = $p > F$. Physiographic data from R. Carignan (GRIL, Université de Montréal, Montréal, QC H3C 3J7, Canada, personal communication).

ter. Finally, the lack of logging impact on the periphyton DM in all lakes or in each cluster may result from an increase of inorganic particle export from watersheds resulting from soil erosion after logging (Likens et al. 1970), which compensated for the decrease in algal biomass.

Few studies have focused on the impact of logging on periphyton biomass in lakes (Rask et al. 1998; Planas et al. 2000) or streams (Shortreed and Stockner 1983; Murphy et al. 1986). Higher benthic algal biomass was observed after logging in all cases except in the stream study of Shortreed and Stockner (1983), where no impact was measured. In streams, the biomass increase was generally explained by an increase of nutrient input or light penetration in the water following logging. Accordingly, in our short-term (1–2 years after logging) study, the decrease in periphyton biomass observed after logging is likely related to the increase in DOC and light extinction coefficient (ϵ_{PAR}) (R. Carignan, GRIL, Université de Montréal, Montréal, QC H3C 3J7, Canada, personal communication). The DOC in our study lakes is coloured and its concentration is highly correlated with ϵ_{PAR} (r^2 = 0.93, $p < 0.0001$; Carignan et al. 2000), i.e., there is less light penetration in the lakes after logging. In addition to DOC, the increased inorganic particle load resulting from erosion after logging, as reflected by the inorganic DM (i.e., DM minus ash-free dry mass (AFDM); not reported), contributes to the attenuation of light availability to periphyton

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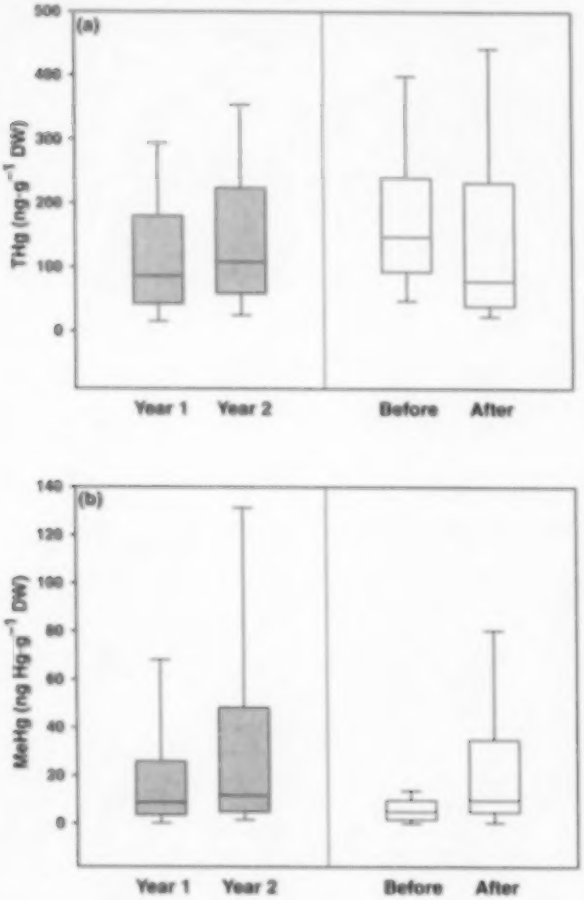
Fig. 5. Box plots showing median values and minimum, 25th, 75th, and maximum percentiles of periphyton (*a*) total mercury concentrations (THg; ng·g^{-1} dry mass (DM)), (*b*) methylmercury concentrations (MeHg; ng Hg·g^{-1} DM) in control lakes (shaded bars; years 1 and 2) and experimental lakes (open bars; before and the first summer after logging).



mats. Generally, a combination of factors can affect periphyton biomass (Fairchild and Everett 1988), and the positive effect of increased nutrient loading should be significant only when light is not limiting (Bourassa and Cattaneo 2000). Accordingly, Murphy et al. (1986) observed a significant increase of periphyton biomass in streams after logging only when the riparian zone was not preserved and, consequently, the amount of light increased in the streambeds.

The differential response of periphyton to watershed logging observed in this study compared with our previous study (Planas et al. 2000) may be explained by variations in runoff. During the first part of this project (1996–1999; Planas et al. 2000), the study area received 10% more precipitation than the annual mean, whereas during this study (i.e., 2000 to 2002), the amount of precipitation was ~15% less than that recorded in the previous 25 years (Environment Canada 2003–2004). Consequently, the short-term nutrient loadings from the watersheds to lakes differed appreciably between the two study periods, and hence, much higher phosphorus concentrations were measured in harvested lakes in 1996–1999 than during the present study (Carignan et al. 2000; Lamontagne et al. 2000). Although periphyton biomass measurements carried out in the 1996–1999 study in control and perturbed lakes were compared with each other, prelogging measurements in the perturbed lakes were not available, and thus, the BACI statistical analysis could not be applied. The BACI approach used in this study provides more conclusive evidence about short-term impacts of watershed disturbance because it accounts for initial conditions (i.e., prelogging) and the natural interannual variability in reference lakes.

In the predictive model, when all lakes and sample years are considered together, the main factor that contributed to the variance of the periphyton algal biomass was latitude ($r^2_{partial}$ = 43%). The periphyton biomass generally increased northward in the boreal Canadian Shield lakes situated in watersheds with low mean watershed slope and more wetlands. After latitude, mean watershed slope was the parameter with the strongest negative correlation with periphyton biomass. It is known that in watersheds with lower mean slopes, the formation of saturated soil profiles is favoured, and thus, the groundwater spends more time in contact with the soil organic layer (D'Arcy and Carignan 1997). Under this scenario, runoff is lower but carries more phosphorus (D'Arcy and Carignan 1997) to the lake. The higher nutrient inputs in lakes with lower watershed mean slopes may account for their higher periphyton biomass.

Impact of logging on THg accumulation in periphyton

Few studies have focused on the THg concentrations in periphyton, but concentrations observed in this study were similar to those reported for periphyton elsewhere (Rask et al. 1994; Cleckner et al. 1998; Roulet et al. 2000). As in the present study, no significant impacts of logging on THg concentrations in periphyton were detected in boreal Finnish lakes (Rask et al. 1994). In the present study, periphyton THg concentrations were negatively correlated with the ratio of wetland area to lake area. This correlation is well documented, at least for natural lakes of the boreal Canadian Shield region, and is explained by wetland retention of THg (St. Louis et al. 1996; Greenfield et al. 2001; Kolka et al. 2001). This mechanism may also account for the lack of logging effect on THg concentrations in periphyton in our study lakes, but we cannot exclude the importance of slow Hg transport in boreal Canadian Shield soils recently highlighted by Hintelmann et al. (2002) using a stable Hg isotope tracer (^{202}Hg). Hintelmann et al. (2002) reported that only 0.25% of their Hg tracer was transferred from the soil to runoff water after 97 days of the addition. Similarly, Aastrup et al. (1991) estimated that under natural conditions, less than 0.1% per year of the Hg and MeHg in an organic-rich soil is transferred to surface waters, with most of the transfer occurring in the spring. Runoff from a watershed normally increases 1 year after logging (Likens et al. 1970), but as noted previously, precipitation and, consequently, runoff in our study area over the period of this investigation (i.e., 2000 to 2002) were lower than recorded in the previous 25 years (Environment Canada 2003–2004). Because both Hg and DOC loading to lakes are believed to be strong functions of runoff (Meili et al. 1991; Kolka et al. 2001), limited

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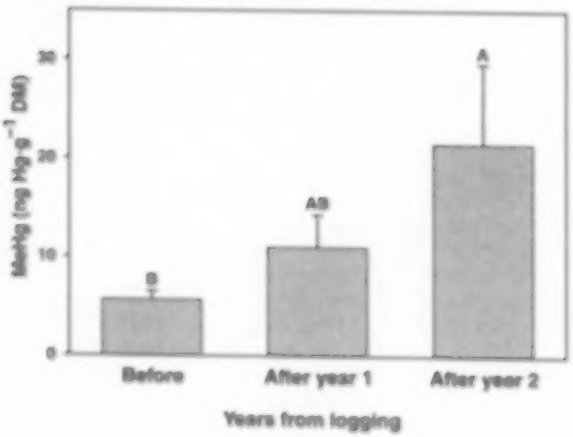
Table 5. The before–after control–impact (BACI) statistical analysis of logging on mercury concentrations in periphyton: (*a*) total mercury (THg; ng·g^{-1} DM) and (*b*) methylmercury (MeHg; ng Hg·g^{-1} DM).

	All lakes		Cluster 1		Cluster 2		Cluster 3		Cluster 4	
Source of variation	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*
(*a*) THg										
Before and after (*B*)	0.04	0.45 ns			0.02	0.29 ns	0.004	0.05 ns		
Impact vs control (*I*)	0.31	3.62 ns			1.64	21.16 *	0.005	0.65 ns		
B·*I*	0.63	7.38 *			0.0003	0.003 ns	0.37	4.95 ns		
$B_{control}$	0.12	2.88 ns			0.01	0.16 ns	0.043	0.71 ns		
B_{impact}	0.07	1.02 ns	0.66	9.32 *	0.0001	0.002 ns	0.24	2.32 ns		
$B_{impact}/B_{control}$		5.50 ns				0.01 ns		5.58 ns		
(*b*) MeHg										
Before and after (*B*)	5.12	30.77 *	1.91	17.16 *	0.46	2.63 ns	0.94	8.31 *	0.12	0.88 ns
Impact vs control (*I*)	1.58	9.49 *	0.07	0.61 ns	0.04	0.22 ns	1.64	16.66 *	0.27	1.94 ns
B·*I*	1.15	6.91 *	0.48	4.31 *	0.61	3.58 *	0.20	2.27 ns	0.10	0.731 ns
$B_{control}$	0.48	4.90 *	0.19	2.42 ns	0.01	0.05 ns	0.098	0.92 ns	0.001	0.011 ns
B_{impact}	5.03	36.46 *	2.87	32.39 *	1.93	12.72 *	0.51	10.01 *	0.22	1.092 ns
$B_{impact}/B_{control}$		10.50 ns		15.10 ns		193.00 *		5.2 ns		220.00 *

Note: MS, mean square error; *F*, Fisher test *F* ratio; DM, dry mass; an asterisk (*) indicates a significant effect; ns indicates a insignificant effect.

Fig. 6. Periphyton methylmercury concentrations (MeHg; ng Hg·g^{-1} dry mass (DM); mean ± standard error) in experimental lakes sampled before and two consecutive summers after logging. Different letters indicate significant mean differences within years (one-way analysis of variance, $p < 0.01$).



precipitation and the presence of wetlands around some of the logged lakes could account for the lack of a clear increase in THg after watershed perturbation due to THg retention in the soils and wetlands.

Impact of logging on MeHg accumulation in periphyton

The weak but nonetheless significant contribution of the percent of watershed logged in the stepwise multiple regression models underscores the deleterious impact of logging on MeHg accumulation by periphyton. The increase in MeHg was particularly high in cluster 2 lakes, even though the percent of perturbation in these lakes (26% and 72%) was within the range of other studied lakes (e.g., cluster 1, 0%–79%). The lakes in cluster 2 are characterized by low mean watershed slopes (<7.0%) and high ratios of wetland area to lake area. The presence of shallow saturated zones in the watersheds of this cluster of lakes should increase the exchange between groundwater and surface runoff water (i.e., limited retention capacity and short residence time of groundwater) and, consequently, enhance the loading of DOC (D'Arcy and Carignan 1997) and MeHg (Meili et al. 1991; Mierle and Ingram 1991). This phenomenon could be amplified by the increase of the water yield after harvesting as a result of decreased whole-tree transpiration and interception (Martin et al. 2000) and the resulting increase of DOC export during exceptional runoff events such as snowmelt (Boyer et al. 2000). Furthermore, although wetlands are known to retain THg, they generally export MeHg (St. Louis et al. 1996). One lake (DF9) in cluster 2 that did not respond to logging was disturbed by intense beaver (*Castor canadensis*) activity before logging. Beaver damming alters the hydrological and chemical properties of the lake, including MeHg concentrations in periphyton (Naiman 1994; Driscoll et al. 1998). Before logging, DOC and total phosphorus (TP) concentrations in this lake were twice as high as in unperturbed lakes of the study area, and high Hg concentrations (mean of 1.5 mg·kg^{-1} wet mass) were found in predatory fish of this lake (R. Carignan, GRIL, Université de Montréal, Montréal, QC H3C 3J7, Canada, personal communication). Beaver activity in lakes often leads to the flooding of the surrounding watershed and promotes the input of chemicals, including Hg, to the aquatic system (Driscoll et al. 1998), a similar response to the increase of chemical export resulting from logging (Lamontagne et al. 2000) or reservoir impoundment (e.g., Mucci et al. 1995). Further studies will be required to confirm these observations as there is a recent resurgence and northern expansion of the beaver population in Canada.

There was no concomitant response to logging on THg and MeHg concentrations in periphyton in lakes. Although

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THg levels were unaffected in the majority of lakes, the MeHg concentrations increased in all but one (DF9) of the logged lakes. The differential impact of logging on THg and MeHg concentrations in periphyton may reflect the bioaccumulation of MeHg produced in the watershed and exported after logging or the greater metabolic activity and affiliated increased Hg methylation potential of the periphyton after logging. Evidence of mercury methylation in periphyton mats has recently been reported in the literature (Guimarães et al. 1998; Cleckner et al. 1999; Desrosiers et al. 2006*a*). Enhanced methylation by periphyton could explain the observed increase (3- to 12-fold) in the ratio of MeHg to THg in five of the logged lakes. The highest absolute MeHg concentration increase was observed in lakes K3 (9.5-fold) and DF7 (8.3-fold) for which 26% and 79% of their respective watersheds were logged. In these lakes, the MeHg concentrations measured after logging accounted for 30% of the periphyton THg pool, whereas the mean for these lakes before logging was 10%. Alternatively, the negative correlation between MeHg and Chl *a* could be interpreted as a bioconcentration of MeHg.

In conclusion, in this before- and after-logging study, we observed a significant decrease in algal biomass (Chl *a*) and increase in MeHg concentrations in periphyton mats in a majority of our harvested watershed lakes (15%–79% logged). The former may be attributed to a decrease in light penetration in lakes after logging. The latter may have a serious impact on organisms of higher trophic levels in small boreal Canadian Shield lakes with relatively large littoral zones (i.e., a mean of 40% of the total surface area in our study). In these lakes, the periphyton communities are generally the dominant primary producers (i.e., (periphyton × area of littoral zone) / (phytoplankton × volume of a pelagic zone) = 0.7 to 20, mean of ~6 in our study lakes). The observed decrease in periphyton biomass combined with the increase in MeHg accumulation may magnify the impact of logging activities on fish and other aquatic predators. Two years after logging, we observed that periphyton MeHg concentrations were still increasing. Long-term monitoring will be necessary to determine how this trend evolves, when MeHg concentrations reach a steady state and (or) return to prelogging values. Similarly, longer-term studies should reveal if the short-term absence of impact on THg concentrations in periphyton persists. An answer to this question is critical because periphyton mats are at the base of the food web. A variation of their relative biomass or an increase of their MeHg concentrations would have a domino effect on the upper trophic levels, particularly in oligotrophic small boreal Canadian Shield lakes. In addition, future work is needed to identify factors that regulate MeHg transport from forested soils to lakes after watershed disturbance, as well as mercury methylation by periphyton under natural and perturbed conditions as a function of watershed characteristics and the extent of disturbance. Before government permits are issued to forestry companies, authorities should seriously scrutinise the watershed morphometric characteristics, particularly its slope. Our study suggests that lakes with watersheds that have mean slopes under 7.0% are much more sensitive to logging, even if a small relative area of the watershed is cut. For example, the MeHg concentration in periphyton of K3 increased 9.5-fold when only 26% of its watershed was logged.

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Appendix A

Table A1. Mean (±standard error) periphyton algal chlorophyll *a* biomass (Chl *a*; mg·m^{-2}), methylmercury (MeHg; ng Hg·g^{-1} dry mass (DM)), and total mercury (THg; ng·g^{-1} DM) concentration in all lakes before and after logging.

(*a*) Natural lakes.

	Chl *a*		MeHg		THg	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
AB35	13.8±1.4	13.4±4.7	**14.9±6.8**	**36.9±17.2**	155.3±45.8	168.1±34.8
AB220	20.2±4.7	24.2±7.5	12.1±3.5	8.0±3.4	—	—
CSL2	89.6±7.2	89.5±23.1	5.1±3.5	4.8±3.6	42.4±21.5	46.28±20.7
CSL5	50.0±4.9	49.9±14.5	9.7±4.6	9.9±4.3	**54.9±15.6**	**100.0±12.3**
DA2	**51.0±4.4**	**34.2±15.9**	8.2±2.8		118.0±22.3	121.7±71.6
DF4	**163.6±19.5**	**51.8±4.0**	**3.1±1.6**	**15.5±5.8**	**157.9±30.6**	**245.3±39.2**
K2	43.3±5.9	70.8±20.8	9.1±2.9	5.3±3.2	107.8±27.0	84.9±30.6
N35	**13.04±4.8**	**23.3±3.1**	25.5±10.3	25.5±4.4	—	—
N55	18.5±2.1	24.9±2.7	10.3±5.5	13.5±4.1	**102.1±15.72**	**217.4±15.1**
N70	28.4±6.5	42.5±18.5	9.8±3.8	17.2±8.7	—	—

(*b*) Perturbated lakes.

	Chl *a*		MeHg		THg	
	Before	After	Before	After	Before	After
DF2	**134.4±25.8**	**72.7±12.6**	**1.7±1.3**	**8.5±3.4**	155.4±36.2	98.8±12.0
DF5	69.5±11.2	58.2±16.6	**5.2±3.1**	**10.7±4.9**	**175.6±60.7**	**53.1±22.9**
DF7	**63.11±7.9**	**24.1±5.9**	**5.4±4.4**	**44.9±16.1**	193.9±43.1	156.2±40.6
DF9	66.5±15.2	41.9±11.7	7.57±4.3	7.3±2.3	83.3±23.9	91.49±16.8

(*c*) Perturbated lakes sampled twice after logging.

	Chl *a*			MeHg			THg		
	Before	After year 1	After year 2	Before	After year 1	After year 2	Before	After year 1	After year 2
K3	**50.2±9.1**	**59.7±14.2**	**30.5±6.6**	**4.3±2.4**	**6.4±2.8**	**41.2±13.0**	**117.7±24.9**	**56.9±12.9**	**300.6±156.8**
K4	**50.3±17.7**	**33.1±8.6**	**29.9±6.5**	**7.7±1.6**	**18.2±3.5**	**28.4±8.9**	**232.9±45.5**	**56.3±12.0**	**230.2±39.9**
K8	98.6±23.4	68.9±8.5	59.7±8.6	6.4±3.2	4.6±2.6	9.2±4.4	**132.3±49.8**	**56.4±19.7**	**239.3±26.5**
DA9	**26.4±13.4**	**60.7±13.7**	**40.8±9.4**	4.2±1.5	15.0±15.3	11.7±8.7	—	—	—

Note: Values in bold indicate lakes with significant differences.

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Stable hydrogen isotopic composition of fishes reflects that of their environment

Gregory W. Whitledge, Brett M. Johnson, and Patrick J. Martinez

Abstract: Otolith microchemistry and isotopic analyses have emerged as effective techniques for providing insights into fish environmental history that are difficult to obtain by other means. Stable hydrogen isotope ratio ($^2H/^1H$ or D/H, expressed as δD) is a possible environmental marker that has not been employed in fish provenance research, although it has been applied as a natural tracer of terrestrial organism migrations. We illustrate the potential of δD to serve as a new natural marker of fish environmental history by demonstrating that significant linear relationships ($r^2 \geq 0.97$) exist between fish otolith and muscle δD and δD of waters that fish inhabit. Differences between mean water δD and both muscle and otolith δD were not significantly correlated with fish total length and were not significantly different among species, indicating that water–fish δD relationships are consistent across fish sizes and species. High r^2 values for regressions of otolith and muscle δD on water δD for fishes inhabiting locations with diverse thermal regimes suggest that relationships between water and fish δD are not strongly affected by water temperature. Demonstration that fish δD clearly reflects water δD provides a foundation for future research to reconstruct fish movement among locations with distinct δD signatures.

Résumé : La microchimie des otolithes et les analyses isotopiques se sont avérées être des techniques efficaces pour obtenir des rétrospectives de l'histoire environnementale des poissons qu'il serait difficile d'avoir autrement. Le rapport des isotopes stables d'hydrogène ($^2H/^1H$ ou D/H, exprimé comme δD) est un marqueur environnemental potentiel qui n'a pas été utilisé dans les études sur l'origine des poissons, alors qu'il a été employé comme traceur naturel des migrations d'organismes terrestres. Nous illustrons le potentiel de δD comme nouveau traceur naturel de l'histoire environnementale des poissons en démontrant qu'il existe des relations linéaires significatives ($r^2 \geq 0{,}97$) entre les δD des otolithes et des muscles et le δD des eaux fréquentées par les poissons. Les différences entre le δD moyen de l'eau et les δD des otolithes et des muscles ne sont pas en corrélation significative avec la longueur totale des poissons et ne diffèrent pas significativement en fonction des espèces, ce qui indique que les relations δD eau–poissons sont uniformes, quelles que soit la taille ou l'espèce de poisson. Les fortes valeurs de r^2 des régressions des δD des otolithes et des muscles sur les δD de l'eau chez des poissons qui vivent dans des régimes thermiques différents laisse croire que les relations entre les δD de l'eau et des poissons ne sont pas fortement affectées par la température de l'eau. La démonstration que les δD des poissons reflètent clairement le δD de l'eau fournit une base pour les travaux futurs afin de récapituler les déplacements des poissons dans des sites possédant des signatures de δD différentes.

[Traduit par la Rédaction]

Introduction

Assays of otolith (calcareous concretions in the inner ear of fishes) trace element and isotopic compositions have emerged as effective techniques for addressing questions regarding fish environmental history that have been difficult to resolve with other approaches (Campana 1999). Otolith aragonite accretes daily in concentric increments that are permanently retained after formation, providing a record of growth over a fish's lifetime (Campana and Thorrold 2001). Trace element and isotopic compositions of otoliths often reflect those of environments occupied by a fish (e.g., Gao and Beamish 1999; Kennedy et al. 2000; Wells et al. 2003) and are unaltered metabolically following deposition (Campana and Thorrold 2001). Thus, association of otolith biochronology with chemical composition enables retrospective description of fish environmental history when an individual has resided in chemically distinct locations for a period of time sufficient to incorporate the signature of those sites (Kennedy et al. 2002). Otolith microchemistry and isotopic analyses have been successfully applied to reconstruct fish thermal history (Joukhadar et al. 2002) and paleoclimates

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(Patterson et al. 1993), indicate metabolic history (Schwarcz et al. 1998), discriminate among stocks (Campana et al. 1994; Gao et al. 2001), detect anadromy (Rieman et al. 1994), describe migration patterns (Secor et al. 2001; Kennedy et al. 2002), and identify natal or larval nursery habitats (Thorrold et al. 1998; Kennedy et al. 2000). The vast majority of published studies employing these methods have been conducted with marine or anadromous species. However, these techniques are rapidly emerging as effective means for reconstructing environmental history of freshwater resident fishes (Bronte et al. 1996; Wells et al. 2003; Brazner et al. 2004).

Thirty-one elements have been detected in otoliths (Campana 1999), although not all will necessarily be applicable to issues of fish provenance in a particular setting. Absence of clear geographic patterns or pronounced temporal variation in elemental or isotopic compositions may limit the ability of some markers to distinguish among geographic locations of interest that may have been occupied by a fish (Gillanders et al. 2001). Additionally, relationships between concentrations or isotopic compositions of some elements in otoliths and the environment can be influenced by temperature, salinity, or fish physiology (Grossman and Ku 1986; Kalish 1989; Gauldie 1996), which may inhibit their utility as natural environmental markers. Therefore, it is often advantageous to examine a suite of chemical markers to maximize the capacity for discriminating among multiple locations where fish have potentially resided (Wells et al. 2003). Addition of new natural markers would be beneficial to expand the applicability and resolution of otolith isotopic and elemental analyses in fish provenance research.

Stable hydrogen isotope ratio ($^2H/^1H$ or D/H, expressed as δD) represents a potential environmental marker that has not been applied in any published studies of fish provenance. However, previous application of δD in research on origins of other migratory organisms, substantial spatial variation in water δD signatures, and presence of hydrogen in fish tissues and otolith proteins collectively suggest that δD has strong potential to serve as a natural marker of fish environmental history. Stable hydrogen isotope analysis has been successfully employed to determine natal origins of North American migratory birds and monarch butterflies (Hobson 1999). Identification of migratory bird origins using δD is based on a continental-scale gradient in δD signatures of precipitation (Hobson 1999) and plant biomass (Yapp and Epstein 1982) that is passed on to higher trophic levels (Cormie et al. 1994). There are also strong correlations between feather hydrogen isotopic composition and growing-season average δD of precipitation at breeding grounds (Chamberlain et al. 1997; Hobson and Wassenaar 1997). Isotopic composition of migrant songbird flight feathers reflects breeding ground signatures because they are grown at or close to those locations and, like fish otoliths, are metabolically inert following synthesis (Mizutani et al. 1990). In addition to continental-scale patterns in precipitation and surface water δD values, stable hydrogen isotopic composition can differ strongly between marine and freshwater environments (Kendall and McDonnell 1998) and often varies substantially among surface fresh waters (Seal and Shanks 1998; Coplen and Kendall 2000) and between surface and ground waters (Krabbenhoft et al. 1994) at smaller spatial scales. Differences in δD among waters make it a useful tracer of water sources for hydrologists (Kendall and McDonnell 1998). Variation in δD among fresh waters is primarily due to continental and altitudinal gradients in precipitation δD and isotopic fractionation associated with evaporation and selective groundwater recharge (Krabbenhoft et al. 1994).

Despite a wealth of knowledge regarding patterns and processes associated with water δD signatures, few published studies have measured stable hydrogen isotopic composition of aquatic biota. Available information indicates that aquatic primary producers are generally depleted in δD by 90‰–110‰ relative to surrounding water and that there is relatively little hydrogen isotopic fractionation associated with trophic transfers (Estep and Dabrowski 1980; Estep and Hoering 1980; Macko et al. 1982). No measurements of δD for fish otoliths have ever been reported, although hydrogen is present in otolith proteins. Herein, we illustrate the potential for hydrogen isotopic composition to serve as a new natural marker of fish environmental history by demonstrating that highly significant correlations exist between fish otolith and muscle δD values and δD signatures of the waters that fish inhabit.

Materials and methods

Eleven species of fishes representing six families (Centrarchidae, Catostomidae, Esocidae, Percidae, Pleuronectidae, Salmonidae) were collected from 11 locations (Table 1) during 2004 by angling, electrofishing, seining, or gill netting. These locations were selected to encompass a broad range of water δD signatures and because fishes living in these locations were known or strongly suspected to have spent all or nearly all of their lives within that same water body. Sampling locations included isolated water bodies in which fishes were known to be naturally reproduced (College Lake, Dixon Reservoir, Bounds Pond, Audubon Pond) and sites where fish were hatched or stocked at age-0 and could not have originated from another source or subsequently traveled elsewhere (Research Hatchery, Highline Reservoir, Blue Mesa Reservoir). Fish may have entered two of our sampling locations from the Colorado River (Government Highline Canal, Horsethief Pond). However, the intake to Horsethief Pond is screened and would only permit passage of larval fishes, while Colorado River and Government Highline Canal δD signatures are indistinguishable (the canal is fed by Colorado River water). We can not rule out the possibility that smallmouth bass (*Micropterus dolomieu*) collected from Lake Powell may have entered the lake from tributaries, but smallmouth bass reproduce in the lake and are not stocked. Pacific halibut (*Hippoglossus stenolepis*) collected near the mouth of Cook Inlet may have spent time in the adjacent Gulf of Alaska.

Five to 23 individuals were collected from each location along with a 20 mL water sample. At seven of the locations, one or two additional water samples were collected during subsequent seasons. Water samples were stored in scintillation vials containing minimal air space and sealed with Parafilm® to curtail evaporative loss and fractionation (Kendall and McDonnell 1998). Total length of each fish was measured to the nearest millimetre, and sagittal otoliths were removed. Otoliths were blotted to remove organic residue,

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Table 1. Locations sampled for fishes, latitude and longitude, species collected, number of individuals collected (n), samples collected, and mean water δD (‰, ±1 standard error (SE)).

Location	Latitude, longitude	Species*	n	Samples†	Water δD (SE)
Government Highline Canal	39°07′N, 108°22′W	WHS	20	O	−124.68 (2.11)
Highline Reservoir	39°16′N, 108°50′W	LMB	5	M, O	−118.97
Blue Mesa Reservoir	38°28′N, 107°15′W	KOK	7	O	−115.36 (0.16)
Lake Powell	37°39′N, 110°31′W	SMB	5	O	−114.00
Horsethief Pond	39°10′N, 108°47′W	BGL, GSF, LMB	7	O	−112.91 (2.67)
Research Hatchery	40°37′N, 105°10′W	CUT	10	M, O	−110.58 (1.24)
College Lake	40°34′N, 105°08′W	LMB, NPK, SMB, YLP	23	M, O	−97.96 (1.19)
Dixon Reservoir	40°33′N, 105°08′W	LMB	8	M, O	−93.33
Bounds Pond	39°05′N, 108°23′W	GSF, LMB	10	M, O	−79.35 (4.24)
Audubon Pond	39°04′N, 108°36′W	BCR, BGL, GSF, LMB	20	M, O	−45.40 (2.27)
Cook Inlet	59°56′N, 152°20′W	HAL	10	M, O	−12.73

Note: $n = 3$ water samples collected during different seasons for Horsethief Pond and Bounds Pond. $n = 2$ water samples collected during different seasons for the Government Highline Canal, Blue Mesa Reservoir, the Research Hatchery, College Lake, and Audubon Pond. $n = 1$ water sample for all other locations.

*BCR, black crappie (*Pomoxis nigromaculatus*); BGL, bluegill (*Lepomis macrochirus*); CUT, cutthroat trout (*Oncorhynchus clarki*); GSF, green sunfish (*Lepomis cyanellus*); HAL, Pacific halibut (*Hippoglossus stenolepis*); KOK, kokanee (i.e., sockeye salmon, *Oncorhynchus nerka*); LMB, largemouth bass (*Micropterus salmoides*); NPK, northern pike (*Esox lucius*); SMB, smallmouth bass (*Micropterus dolomieu*); WHS, white sucker (*Catostomus commersonii*); YLP, yellow perch (*Perca flavescens*).

†M, muscle; O, otolith.

rinsed with distilled water, and air-dried. Dorsal muscle plugs were removed from fishes collected at 7 of the 11 sampling locations, frozen on the date of capture, and stored at −10 °C. Muscle tissue samples were dried at 60 °C for 72 h; muscle and otolith samples were ground to a flour-like consistency with a mortar and pestle.

Water, otolith, and muscle samples were analyzed for hydrogen isotopic composition using a high temperature conversion elemental analyzer interfaced with a Thermo Finnigan Delta Plus XL® isotope ratio mass spectrometer (Thermo Electron Corp., Waltham, Massachusetts) in the Water and Environmental Research Center at the University of Alaska-Fairbanks. Hydrogen isotope ratios are reported in standard δ notation, defined as the per mil deviation between isotope ratios of a sample and standard (Vienna Standard Mean Ocean Water; Krabbenhoft et al. 1994):

$$(1) \qquad \delta D(‰) = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where R represents $^2H/^1H$. Mean coefficient of variation among replicate measurements was 0.7% for water samples and 0.9% for solid samples.

Least-squares linear regression was applied to relate both mean otolith and mean muscle δD values to corresponding mean water δD values from our sampling locations. Bonferroni joint confidence intervals were used to test whether regression models had a y intercept of zero and a slope of one. Assessment of possible effects of fish size and species on relationships between water and both otolith and muscle δD values was also of interest. However, regressions indicated that water–otolith and water–fish muscle differences in δD were a function of water δD signature. Therefore, otolith and muscle δD values for each fish were standardized to the mean water δD value of all sampling locations combined (−85.8‰) using regression equations described above. Standardized differences between mean water and fish (otolith and muscle) δD values were then regressed on fish total length (mm) to assess the influence of fish size on relationships between water and fish δD values. Standardized differences between mean water and fish (otolith and muscle) δD values were also compared among species using analysis of variance (ANOVA) followed by the Bonferroni multiple comparison method for separation of means.

Results

Highly significant ($P < 0.05$) linear relationships were observed between both mean otolith and mean muscle δD values and mean water δD signatures at our sampling locations (Fig. 1). All otolith and muscle samples were depleted in deuterium (^2H) with respect to corresponding water δD signatures. Bonferroni joint 95% confidence intervals indicated that slopes of regressions of mean otolith and mean muscle δD values on mean water δD signatures were significantly less than one and y intercepts of these regression models were significantly less than zero. Slopes of regression relationships between otolith and water δD (0.50 ± 0.05 standard error (SE)) and muscle and water δD (0.49 ± 0.04 SE) were not significantly different from one another (heterogeneity of slopes test, $P > 0.1$), although mean muscle δD values were depleted in ^2H by an average of 31.43‰ (±1.96‰ SE) with respect to corresponding mean otolith δD values.

Standardized differences between mean water δD and both muscle and otolith δD values for individual fish were not significantly correlated with fish total length (Pearson's correlation coefficients, $P > 0.1$, $n = 119$ otolith samples, $n = 80$ muscle samples). Fish total lengths ranged from 39 to 1092 mm. Standardized differences between mean water δD and both mean otolith and mean muscle δD were also not significantly different among the 11 species included in this study (ANOVA, $P > 0.1$, $n \geq 5$ for each species; $1 - \beta > 0.9$ for otolith data, $1 - \beta = 0.8$ for muscle data for the largest observed effect sizes).

Discussion

Significant linear relationships between both mean fish otolith and mean muscle δD values and mean water δD sig-

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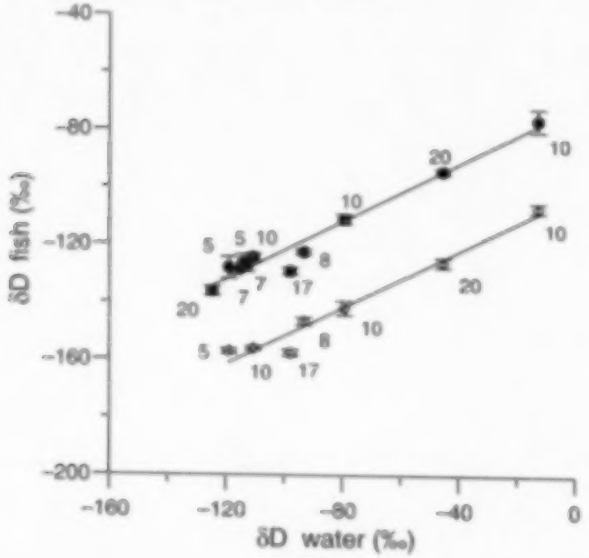
Fig. 1. Mean otolith and muscle δD values in relation to mean water δD signatures. Solid circles represent means of otolith samples from each location ±1 standard error (SE); open diamonds represent means of corresponding muscle samples ±1 SE. Sample sizes are indicated next to each data point. Solid lines indicate least-squares linear regression functions fit to otolith data ($y = 0.50x - 71.35$; $r^2 = 0.97$, $P < 0.0001$) and muscle data ($y = 0.49x - 102.53$; $r^2 = 0.98$, $P < 0.0001$).



nature with coefficients of determination >0.97 suggest that stable hydrogen isotopic composition in water has a strong influence on fish δD and that stable hydrogen isotopes have great potential to serve as a new natural marker of fish environmental history when fish move among locations with distinct δD signatures. Water δD varies substantially among non-oceanic surface waters and between surface and ground waters, often at spatial scales conducive to tracking movements of fishes among water bodies (Seal and Shanks 1998; Coplen and Kendall 2000). Although δD of surface waters may change seasonally (Krabbenhoft et al. 1994), differences in δD among connected aquatic environments can persist when spatial variation in water δD exceeds temporal variability within environments (Coplen and Kendall 2000; G. Whitledge, unpublished data). We expect that δD values of discrete otolith growth bands will reflect water δD signatures at the time of their deposition, as do isotopic compositions of other elements (e.g., $\delta^{18}O$, $^{87}Sr/^{86}Sr$) that have been successfully applied in studies of fish provenance (Gao and Beamish 1999; Kennedy et al. 2002) and δD values of migratory bird flight feathers (Hobson 1999). The 1–2 mg sample size requirement for stable hydrogen isotope analysis of otoliths by bulk analysis using isotope-ratio mass spectrometry may limit the resolution of δD as a natural marker of fish environmental history; advancement of microsampling techniques for δD analysis such as ion microprobe technology (Weber et al. 2002) would enhance the utility of δD as an environmental tracer for fishes. Application of muscle δD to issues of fish environmental history will require knowledge of metabolic turnover rates to establish time frames over which isotopic assays will integrate, as with isotopes of other elements (Hesslein et al. 1993). Analysis of multiple tissues with different turnover rates will likely prove useful for tracking movements of individuals, as has been the case for other isotopes (Hobson 1999).

Strong correlations between both otolith and muscle δD and δD of waters inhabited by fishes were evident despite lack of correction of otolith and muscle samples for hydrogen exchange. Non-carbon-bonded hydrogen (e.g., O—H and N—H) in organic matter may potentially exchange with ambient water, including laboratory water vapor that can vary temporally and geographically (Schimmelmann 1991; Cormie et al. 1994). For otolith protein (otolin), about 39% of the hydrogen is potentially exchangeable based on its amino acid composition, which is quite similar among fish species (Degens et al. 1969). Estimated proportions of nonexchangeable and potentially exchangeable hydrogen in otolin are similar to those in bird feather keratin (40% potentially exchangeable hydrogen; Hobson 1999); otolin strongly resembles keratin in amino acid composition (Degens et al. 1969). Although 40% of hydrogen in feather and whale baleen keratin is theoretically exchangeable, only about 15% effectively exchanges with ambient water vapor (Chamberlain et al. 1997; Wassenaar and Hobson 2003), suggesting that readily exchangeable hydrogen in otolin may be less than the 39% that is theoretically exchangeable. Mean proportion of exchangeable hydrogen was 19.3% (±0.5% standard deviation) for quail muscle tissue (Wassenaar and Hobson 2000); proportion of exchangeable hydrogen in fish muscle tissue is unknown. Slopes of regressions of otolith and muscle δD on water δD may be indicative of the proportion of nonexchangeable hydrogen (acquired from the environment) in our samples if the ambient laboratory water vapor possessed a constant δD value. Highly significant relationships between both otolith and muscle δD and δD of waters inhabited by fishes strongly suggest that our otolith and muscle samples had equilibrated with ambient laboratory water vapor δD that did not change substantially during the time period of our analyses (Hobson 1999). We note that the 95% confidence interval for the slope of the regression of otolith δD on water δD includes 0.6, a figure very close to the predicted 61% nonexchangeable hydrogen in otolin. Additional evidence of constant laboratory water vapor δD is provided by mean δD values for four batches of otoliths collected on 30 March 2004 from one sampling location (Government Highline Canal) and analyzed during different months (February, August, and October 2004; March 2005). Mean δD values for these batches of samples were not significantly different (ANOVA, $P = 0.47$), indicating that any hydrogen exchange with ambient laboratory water vapor was not differentially affecting δD values of samples analyzed on different dates (i.e., any temporal variation in water vapor and exchangeable hydrogen δD was insufficient to cause differences among samples analyzed during this study). Differences in δD among otolith or muscle samples therefore reflected differences in δD of nonexchangeable hydrogen. Stable isotopic composition of nonexchangeable hydrogen in metabolically inert otoliths will provide a permanent record of δD signatures from environments occupied by fish. Although our δD data are internally comparable, future applications of δD assays of fish otoliths and tissues should report

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results for nonexchangeable hydrogen only, using methods recently developed for bird feathers and other complex organic materials (Wassenaar and Hobson 2000, 2003). Reporting δD data in this manner will facilitate comparison of results among laboratories (Hobson 1999).

Depletion of δD values for fish otolith and muscle samples relative to water hydrogen isotopic signature is consistent with published studies that have demonstrated lower δD values in aquatic biota compared with the water they inhabit (Estep and Dabrowski 1980). Twenty to thirty percent of hydrogen in quail tissues is derived from drinking water, with the rest derived from the diet (Hobson et al. 1999); the relative contribution of water and diet to hydrogen in fishes is unknown. Consistently lower δD values for muscle samples compared with otoliths from fish collected at a given location may be related to the presence of lipids in muscle samples; lipids are typically depleted in ^{2}H compared with proteins (Smith and Epstein 1970; Estep and Hoering 1980). Further experimental research to refine our understanding of sources and behavior of stable hydrogen isotopes in fishes and other aquatic food web components is warranted.

Relationships between both fish muscle and otolith δD and water δD encompass a wide range of water δD signatures and appear to be consistent across distantly related fish species and a wide range of fish sizes. High r^2 values for linear regressions of fish otolith and muscle δD on water δD were observed despite inclusion of fishes from locations with diverse thermal regimes (mean maximum water temperatures ranged from 10.1 to 27.4 °C (G. Whitledge, unpublished data); research hatchery fish were held at 12 ± 1 °C (P. Schler, Colorado Division of Wildlife Research Hatchery, Box 96, Bellvue, CO 80512, USA, personal communication)), suggesting that the relationship between water and fish δD values is not strongly affected by water temperature as is otolith $\delta^{18}O$. Thus, δD may provide a valuable alternative to otolith $\delta^{18}O$ analysis for discriminating among locations in which distinct thermal regimes prevent spatial differences in water $\delta^{18}O$ from being expressed in otoliths. The discovery of highly significant relationships between water and fish δD provides a foundation for stable hydrogen isotope ratios to serve as a valuable additional tool in research directed at reconstructing fish environmental history.

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Route selection in a large river during the homing migration of Chinook salmon (*Oncorhynchus tshawytscha*)

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Abstract: Upstream-migrating adult salmon must make a series of correct navigation and route-selection decisions to successfully locate natal streams. In this field study, we examined factors influencing migration route selections early in the migration of 4361 radio-tagged adult Chinook salmon (*Oncorhynchus tshawytscha*) as they moved upstream past dams in the large (~1 km wide) Columbia River. Substantial behavioral differences were observed among 11 conspecific populations, despite largely concurrent migrations. At dams, Chinook salmon generally preferred ladder passage routes adjacent to the shoreline where their natal tributaries entered, and the degree of preference increased as salmon proximity to natal tributaries increased. Columbia River discharge also influenced route choices, explaining some route selection variability. We suggest that salmon detect lateral gradients in orientation cues across the Columbia River channel that are entrained within tributary plumes and that these gradients in cues can persist downstream for tens to hundreds of kilometres. Detection of tributary plumes in large river systems, using olfactory or other navigation cues, may facilitate efficient route selection and optimize energy conservation by long-distance migrants.

Résumé : Les saumons adultes en migration vers l'amont doivent prendre une série de décisions précises de navigation et de sélection de parcours afin de réussir à trouver leur cours d'eau d'origine. Notre étude de terrain examine les facteurs qui influencent le choix de la route de migration tôt dans le processus chez 4361 saumons chinook (*Oncorhynchus tshawytscha*) adultes munis d'étiquettes radio alors qu'ils se déplacent vers l'amont à travers des barrages dans la partie large (~1 km de largeur) du Columbia. On observe d'importantes différences dans le comportement des 11 populations conspécifiques, même si les migrations sont en grande partie simultanées. Au niveau des barrages, les saumons quinnat préfèrent généralement les routes avec échelles à poissons situées près de la rive sur laquelle aboutit leur tributaire d'origine et cette préférence est d'autant plus grande que les saumons s'approchent de leurs cours d'eau de naissance. Le débit du Columbia affecte aussi le choix des parcours, ce qui explique une partie de la variation de la sélection des routes de migration. Nous émettons l'hypothèse selon laquelle les saumons sont capables de déceler les gradients latéraux de signaux d'orientation en travers du chenal du Columbia; ces signaux se forment dans les panaches de diffusion des tributaires et peuvent persister vers l'aval sur des distances de dizaines ou de centaines de kilomètres. La détection des panaches formés par les tributaires dans les grands systèmes fluviaux, par l'intermédiaire de signaux de navigation olfactifs ou autres, peut ainsi faciliter la sélection efficace des parcours et optimiser la conservation d'énergie chez les migrateurs de longue distance.

[Traduit par la Rédaction]

Introduction

Long-distance migrations have fascinated naturalists for centuries. Especially intriguing are the migrations of those species that return to natal breeding grounds after movements over thousands of kilomtres (Dingle 1996), including sea turtles (e.g., *Chelonia mydas*, Åkesson et al. 2003), shorebirds (Battley et al. 2004), albatross (e.g., *Phoebastria* spp., Hyrenbach et al. 2002), and anadromous salmonids (*Oncorhynchus* and *Salmo* spp.; Groot and Margolis 1991; Quinn et al. 1999). Ocean migrations by salmonids can cover thousands of kilometres over several years, followed by precise freshwater homing migrations of 1500 km or more to inland spawning grounds (Milligan et al. 1985; Waples et al. 2001). These migrants must balance the physiological demands of migration with precise spatial and temporal homing requirements.

In fresh water, return migration by anadromous adult salmonids appears to be driven largely by chemically based olfactory cues (Scholz et al. 1976), particularly in small

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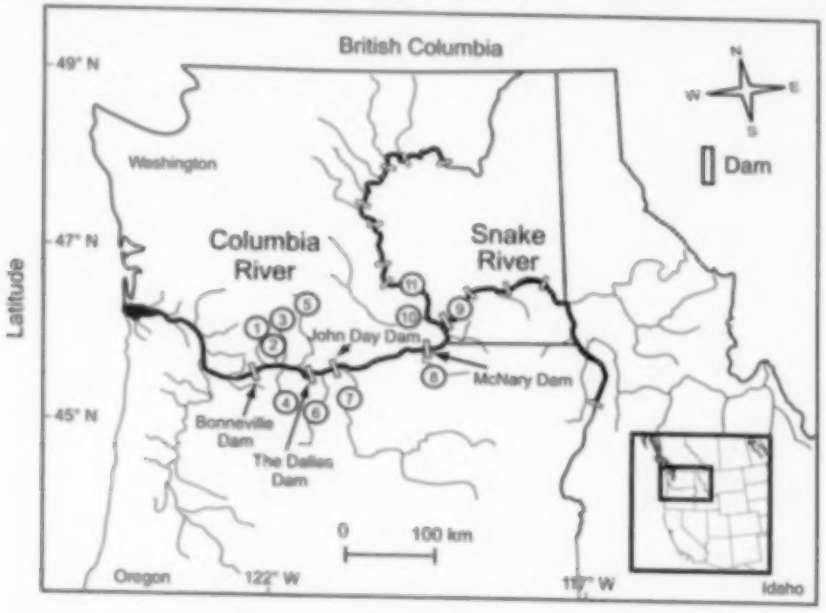
Fig. 1. Columbia River Basin (Pacific Northwest, USA — inset) accessible to migrating adult Chinook salmon (*Oncorhynchus tshawytscha*), including tributaries where radio-tagged fish were last recorded and four lower river mainstem dams where passage routes were monitored. Tributary rivers: (1) Wind, (2) Little White Salmon, (3) White Salmon, (4) Hood, (5) Klickitat, (6) Deschutes, (7) John Day, (8) Umatilla, (9) Snake, (10) Yakima, and (11) upper Columbia.



rivers and coastal streams. Returning fish use chemosensory memories from site-specific imprinting to natal stream odors as juveniles and memories from additional outmigration sites during the transformation from fresh to marine waters (Dittman and Quinn 1996; McCormick et al. 1998). The reverse sequence of these olfactory signposts (Harden Jones 1968; Quinn et al. 1989) presumably guides adult fish migrating to spawning grounds and facilitates discrimination among alternate routes as fish proceed up river corridors. The chemical and olfactory components of adult orientation and homing have been demonstrated experimentally in the laboratory (e.g., Shoji et al. 2003) and in the field at scales of tens to hundreds of metres (Johnsen and Hasler 1980; Hasler and Scholz 1983). Little is known, however, about how salmonids and other aquatic organisms orient and home in larger-scale natural systems (e.g., tens to hundreds of kilometres; Vickers 2000).

In this study, we examined route selection behaviors by 11 conspecific spring–summer Chinook salmon (*Oncorhynchus tshawytscha*) populations during adult return migration through the very large Columbia River migration corridor. Most study fish were stream-type Chinook salmon, though some summer migrants were ocean- or reservoir-type (Brannon et al. 2004). The river has the second highest mean annual discharge of all US rivers (approximately 244 billion $m^3 \cdot year^{-1}$ at the mouth) and historically produced some of the largest salmon and steelhead runs in North America (Chapman 1986). The Columbia main stem has been extensively developed for hydroelectric production, resulting in a series of large run-of-river impoundments. Lower river reservoirs (Fig. 1) average 1–2 km in width and 10–40 m in depth, with current velocities that vary widely with season and volume (Scheibe and Richmond 2002; US Army Corps of Engineers 2002). Many anadromous migrants, including dozens of reproductively isolated spring–summer Chinook salmon stocks (Myers et al. 1998; Brannon et al. 2004), pass through long sections (e.g., hundreds of kilometres) of this altered migration corridor en route to natal spawning grounds in upstream tributaries. Most returning salmon must pass one to four lower Columbia River dams and reservoirs, and many pass up to five additional dams and reservoirs to reach spawning tributaries. Each of the four lower river dams has two principal passage routes for upstream-migrating fish — one fish ladder near each shoreline — and these routes are readily monitored. This configuration creates a unique opportunity to examine migration behaviors, test hypotheses about salmon route selection, and examine potential orientation mechanisms in a large river.

We used a large radiotelemetry data set developed over multiple years (described in Keefer et al. 2004*a*) to test two general hypotheses: (1) whether patterns in ladder use among stocks were nonrandom, and if so, (2) whether salmon route selections at dams were consistent with downstream detection of and orientation to natal tributaries. We expected returning adult Chinook salmon would use olfactory or other cues to orient to plumes of natal tributaries, aggregating along the side of the Columbia River downstream from natal river confluences. Such aggregation on the "correct" shoreline would be reflected in stock-specific differences in fish ladder use at dams. If true, salmon returning to south shore tributaries would have a higher probability of using south shore fish ladders, and vice versa. We also expected the

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Table 1. Mean seasonal discharge ($m^3 \cdot s^{-1}$, April–July) of study tributaries and number of radio-tagged adult Chinook salmon (*Oncorhynchus tshawytscha*) last recorded at each tributary site, 1996–2002.

Tributary (map number)	Mean (range) discharge ($m^3 \cdot s^{-1}$)	No. of Chinook salmon						
		1996	1997	1998	2000	2001	2002	Total
Wind (1)	29 (8–56)	93	53	59	100	25	59	389
Little White Salmon (2)	15 (9–25)	99	24	15	39	10	39	226
White Salmon (3)	37 (22–44)	17	9	2	2	2	2	34
Hood (4)	28 (13–40)	4	10	3			2	19
Klickitat (5)	56 (27–72)	20	28	26	14	3	3	94
Deschutes (6)	168 (126–217)	68	46	74	110	10	30	338
John Day (7)	99 (8–166)	37	7	28	26	7	22	127
Umatilla (8)	15 (14–42)	15	12	9	11	3	16	66
Snake (9)	2488 (811–3832)	122	317	247	210	359	285	1540
Yakima (10)	127 (39–193)	33	19	41	46	88	72	299
Upper Columbia (11)	5371 (2447–7777)	130	224	262	256	156	201	1229
Total		638	749	766	814	663	731	4361

Note: Discharge estimates were assembled from daily mean US Geological Survey data; mean = 71 years-site^{-1}.

degree of preference would depend on the distance to the tributary confluence and on discharge volume from tributaries and in the main stem, because tributary plumes should remain hydrologically distinct for greater distances downstream for large tributaries and under higher flow conditions (Fischer et al. 1979; Rutherford 1994). Dam operations also potentially influence route selections, both through turbulent mixing and disruption of plumes and through the distribution of attractive flows downstream. Discharge volumes from Columbia River dam fishways are generally constant to maintain suitable hydraulic conditions in the ladders, but both the volume and distribution of river discharge passed through turbines and over spillways varies widely.

Orienting to the natal shoreline prior to reaching natal tributary confluences could have fitness consequences, because such behavior would reduce energetic losses and mortality associated with searching for natal sites, including fallback over dams that occurs when salmon overshoot natal sites (Boggs et al. 2004). Alternatively, a lack of preference for natal shore ladders would suggest individuals use homing mechanisms other than lateral gradients in olfactory cues. For instance, if homing cues are rapidly mixed across the river channel, we expected orientation to tributaries to occur over relatively short spatial scales near the tributary–mainstem confluences, resulting in no ladder bias at downstream dams.

Materials and methods

Salmon collection and radio tagging

Adult spring–summer Chinook salmon returning to the Columbia River basin were collected at Bonneville Dam (river kilometre 235) in an adult fish trap adjacent to the north shore fish ladder (Fig. 1). During 6 study years, we collected 5526 spring–summer Chinook salmon, of which 4361 (79%) returned to tributaries upstream from Bonneville Dam or to the upper Columbia Basin (Table 1). Typical fish were ages four or five and had body lengths between 75 and 85 cm. Samples averaged 0.78% of the spring–summer Chinook salmon counted passing Bonneville Dam each year between 1 April and 31 July (US Army Corps of Engineers 2002). Because many Columbia River Basin Chinook salmon populations are listed as threatened or endangered (National Marine Fisheries Service 2000), special collection permits were needed and federal, state, and university animal handling and care protocols were strictly followed.

Salmon were sampled approximately in proportion to daily run counts (as in Keefer et al. 2004*b*), though we tended to under-sample during count peaks and over-sample during count nadirs. During 1996–1998, salmon were non-selectively collected as they passed through the trap facility during each sampling day. Random sampling was not possible because only fish passing via the north shore ladder could be collected, and no fish were sampled at night. In 2000–2002, we modified our sampling strategy to select for fish with passive integrated transponder (PIT) tags that identified natal sites (Prentice et al. 1990). Use of previously PIT-tagged fish (37% of the 2000–2002 samples) resulted in larger sample sizes for some stocks (see Keefer et al. 2005), but these subsamples represented less than 0.5% of the annual runs and should not affect interpretation of the basic behavioral results presented here, which are based on individual fish route selections.

Protocols for fish trapping, anesthetization (methane sulphonate or clove oil), handling, and intragastric insertion of uniquely coded radio transmitters were the same in all years and are described in Keefer et al. (2004*c*). Salmon recovery from anesthesia occurred in a dark, 2275-L, oxygenated transport tank full of river water. Most fish regained equilibrium within a few minutes, but were held an average of 1.6 h to ensure recuperation. Following recovery, fish were randomly released about 10 km downstream from Bonneville Dam at sites on both sides of the Columbia River in similar percentages (49% from Oregon shore). Total fish mortality was less than 1% during tagging, recovery, and release (Keefer et al. 2004*a*).

Stock identification and route monitoring

Chinook salmon were identified as belonging to one of 11 tributary-specific stocks (including the upper Columbia River Basin) based on final recorded fish locations (Table 1).

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Identifications were derived primarily from telemetry records at aerial antennas deployed in each major Columbia River tributary and from aerial and underwater antennas at Snake River and upper Columbia River dams (Fig. 1). These data were supplemented with location data collected using mobile antennas attached to boats and trucks and from recoveries of transmitters from hatcheries, tributary spawning ground surveys, and fisheries. Stock identifications should have been accurate given the breadth of our monitoring effort, although some salmon likely strayed into non-natal rivers or were misclassified based on ambiguous telemetry data. During the years 2000–2002 when known-source, PIT-tagged fish were present in the samples, there was 97.8% agreement between stock assignment based on juvenile PIT-tagging location and final telemetry records among PIT-tagged fish. This agreement suggests that bias due to straying was minor. We note that study tributaries included multiple spawning populations, and finer-resolution stock classifications are possible (e.g., Nehlsen et al. 1991).

Fish behavior at the four lower Columbia River dams (Bonneville, The Dalles, John Day, McNary) was monitored with an extensive collection of aerial and underwater antennas in tailraces, fishways, and fish ladders. Radio-tagged salmon could approach dams and enter fishways via many routes, and most fish moved between multiple sites at each dam before selecting and passing a fish ladder (e.g., Keefer et al. 2003). Each dam had two ladders, one immediately adjacent to each shoreline (except the south ladder at Bonneville Dam, which was situated on an island close to the south shoreline). Exits from the tops of these ladders were monitored with highly efficient underwater antenna arrays (mean detection rates >98%). Passage route selections at individual dams were assigned based on the first ladder each fish passed. A small number of passage events (<3%) occurred through navigation locks of dams, and these events were included with the total of the adjacent shoreline. Subsequent passages, which occurred after some salmon fell back over a dam (see Boggs et al. 2004), were excluded, as were passage events that could not be attributed to a specific ladder. The first ladder passage was selected for analyses because all fish were presumably naïve regarding routes (except at Bonneville Dam) and because of the potential for injury during fallback events to affect orientation behavior.

Statistical analyses

Adult Chinook salmon orient to shorelines during upstream migration (e.g., Hughes 2004). Consequently, we examined the possibility that release site downstream from Bonneville Dam would be associated with ladder use at Bonneville Dam ~10 km upstream. Chi-square tests comparing frequencies of ladder use versus release shore tested whether ladder use deviated significantly from a 50:50 expectation for each stock. For these tests, we combined frequencies across all years, though single-year comparisons yielded similar results (M.L. Keefer, C.C. Caudill, C.A. Peery, and T.C. Bjornn, unpublished data).

We assessed salmon route selections at all four lower Columbia River dams using replicated goodness-of-fit tests (*G* tests; Sokal and Rohlf 1995) that compared salmon passage frequencies between ladders and years for each stock at each dam. We treated year as the replicate and generated expected frequencies for each year using two null hypotheses. First, we assumed salmon routes would be random at each dam (i.e., ladder use would be 50:50 south shore:north shore). However, counts of salmon at dams indicate clear differences in ladder use among dams within season and among years (see Results; Fig. 2). These differences are likely due to differences in river conditions, dam configuration and operations, and stock composition. Therefore, we also generated expected frequencies using the daily ladder counts for all adult spring–summer Chinook salmon from both ladders at dams during the dates when 90% of the radio-tagged fish from each stock passed each dam in each year. The *G* tests provided tests of both deviations from overall expected proportions for each ladder across all years and simultaneously tested for significant interannual variation in proportions within stock (Sokal and Rohlf 1995). The *G* tests determined whether the radio-tagged sample of each stock differed in ladder use from the overall population that passed each dam during the same period. Such tests were conservative, because each stock was a subpopulation of the total ladder counts during each period, and potential influence of each stock on the expected frequencies should differ based on the relative run size of each stock (i.e., the type II error for relatively large stocks will be greater than that for smaller stocks because large stocks have a greater effect on counts at dams and the expected frequencies).

We also examined the potential for interannual variation in flow to influence ladder use. For each stock at each dam, we regressed the annual proportion of salmon passing via the natal shore ladder against the mean discharge encountered by radio-tagged fish in each year. Discharge and ladder count data were provided by the US Army Corps of Engineers.

Results

Effects of release site

Comparisons between the release site (Washington versus Oregon shore) of radio-tagged fish and individual ladder use at Bonneville Dam revealed that many fish crossed the river channel at least one time. Overall, 49.2% ($n = 2134$) individuals passed on the same side as the release site and 50.8% ($n = 2207$) individuals passed on the shore opposite their release site. Within stock, release location was not associated with subsequent ladder passage at Bonneville Dam in 9 of 11 comparisons. The Klickitat River fish were more likely to pass on the same shore as they were released (63.8%; $P = 0.0058$, $n = 94$). Snake River fish were more likely to pass via the Oregon shore ladder (52.0% vs. 48.0%; $P = 0.0089$, $n = 1530$), a pattern resulting primarily from a higher than expected percentage of fish released from the Washington shore passing at the Oregon shore ladder (28.4%).

Salmon route selection at dams

Daily percentages of all adult spring–summer Chinook salmon counted by the US Army Corps of Engineers passing the four lower Columbia River dams varied widely within years and between years and dams (Fig. 2). Overall, more salmon used ladders near the south shoreline at The Dalles and John Day dams in almost all years and favored ladders near the north shoreline at Bonneville Dam in 2001 and

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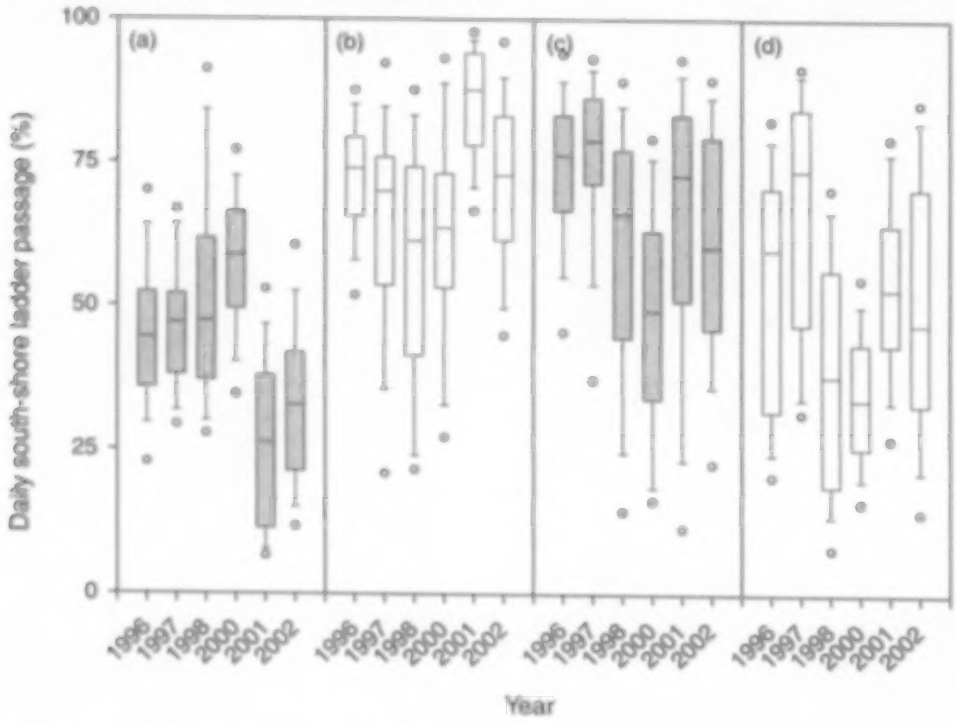
Fig. 2. Daily percentages (box plot showing median, quartile, 5th, 10th, 90th, and 95th percentiles) of all adult spring–summer Chinook salmon (*Oncorhynchus tshawytscha*) counted passing dams that used ladders near the south shoreline at (*a*) Bonneville, (*b*) The Dalles, (*c*) John Day, and (*d*) McNary dams from April through July during the 6 study years. Data provided by US Army Corps of Engineers (T. Hurd, USACE, P.O. Box 2946, Portland, OR 97208, USA).



2002. Daily ladder use proportions were most variable at McNary Dam. Stock-specific ladder use by radio-tagged salmon also varied (Fig. 3), with significant (G tests: $P < 0.05$) between-year differences in 13 of 25 dam × stock combinations (Table 2). As expected, tests of the first null hypothesis (expected ladder passage proportions = 50:50) showed that stock-specific ladder use was not random (Table 2). This was especially true at the three upstream dams, where proportions were significantly different from random for 12 of 14 pairs. At Bonneville Dam, just upstream from the release sites, ladder use differed from 50:50 only for Hood River salmon (Table 2, Fig. 3).

Tests of the second null hypothesis (expected ladder passage proportions based on salmon counts at ladders) indicated that many radio-tagged stocks preferentially used dam passage routes adjacent to shorelines where their natal tributaries entered the main river. As with the 50:50 tests, significant departures from expected route selections among stocks were less frequent at Bonneville Dam and progressively more frequent at upstream dams (Table 2). After accounting for between-year differences, salmon stocks selected ladders adjacent to natal shorelines at significantly (G test $P < 0.05$) higher than expected proportions in 13 of 25 dam × stock combinations (Table 2). Four dam × stock combinations (Little White Salmon, Yakima, and upper Columbia stocks at Bonneville Dam and upper Columbia stock at The Dalles Dam) passed via a natal shoreline ladder at lower than expected rates ($P < 0.05$).

Proximity to confluences and river discharge

Biases in favor of dam passage routes adjacent to natal shorelines increased with the size of the natal tributary and the proximity of dams to each tributary's confluence with the Columbia River. On average, annual ladder biases (observed – expected from null hypothesis 2) were higher for stocks returning to the high-discharge upper Columbia and Snake rivers than for stocks returning to the other nine tributary sites (Fig. 4). Biases averaged +14.4% for the upper Columbia and Snake river stocks at McNary Dam, which is the first dam downstream (52 km) from the upper Columbia – Snake river confluence. Average biases for these two stocks dropped to +6.8% at John Day Dam (two dams and 175 km downstream from the confluence) and were not distinguishable from zero at The Dalles Dam (214 km downstream) and Bonneville Dam (287 km downstream). On average, stocks from the other, smaller tributaries had natal shoreline biases of +6.5% at the dams immediately downstream from their respective confluences and +6.4% two dams downstream. Biases were negative three and four dams downstream (Fig. 4). Exceptions among the stocks from smaller tributaries included the John Day River stock at John Day Dam (confluence 4 km upstream from the dam, bias = +29.2%), the Yakima River at McNary Dam (70 km, +16.0%), and the Deschutes River at The Dalles Dam (20 km, +10.0%) (Fig. 3). These patterns suggest that salmon were more likely to aggregate near the Columbia River shoreline of the natal tributary confluence during migration

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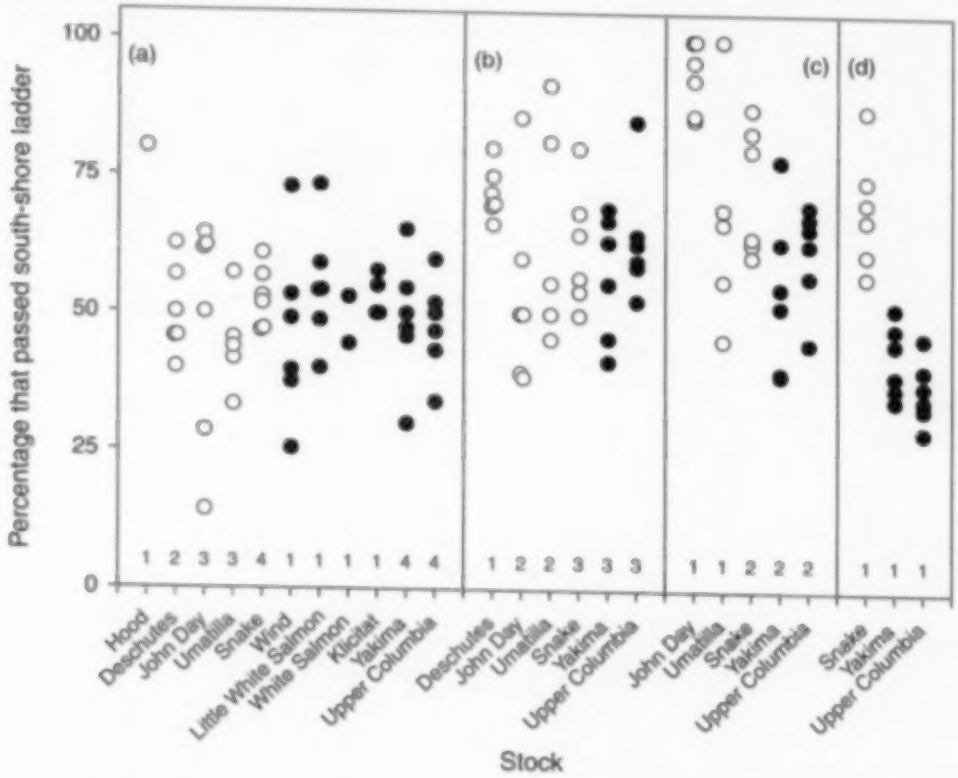
Fig. 3. Annual percentages of radio-tagged Chinook salmon (*Oncorhynchus tshawytscha*) stocks recorded passing south shore fish ladders at (*a*) Bonneville, (*b*) The Dalles, (*c*) John Day, and (*d*) McNary dams after release downstream from Bonneville Dam. Open circles (○) are for stocks from south shore tributaries; solid circles (●) are for stocks from north shore tributaries. Numbers above stocks show the number of dams downstream from natal tributary confluences (i.e., Bonneville Dam is one dam downstream from the Hood River in panel *a*). Stock years with $n < 5$ were excluded.



as they neared the confluence and that aggregation began at greater distances downstream when natal rivers were large.

Interannual differences in ladder use for some, but not all, stocks were associated with mean annual discharge during the period each stock passed, which ranged from about 3000 to 14 000 $m^3 \cdot s^{-1}$. At McNary Dam, for example, annual use of the south shore ladder by Snake River salmon was positively correlated with mean encountered discharge ($r = 0.930$, $P = 0.007$, df = 4), as was use of the north shore ladder by upper Columbia River salmon ($r = 0.775$, $P = 0.070$, df = 4) (Fig. 5). Similar correlations were observed at John Day Dam for John Day River ($r = 0.880$, $P = 0.021$, df = 4), Umatilla River ($r = 0.907$, $P = 0.013$, df = 4), and Snake River ($r = 0.792$, $P = 0.006$, df = 4) stocks. No correlations were significant ($P > 0.10$) for any stocks at Bonneville or The Dalles dams, for Yakima River salmon at John Day and McNary dams, or for upper Columbia River salmon at John Day Dam.

Discussion

In many cases, the radio-tagged Chinook salmon in this study demonstrated nonrandom ladder use that differed among stocks in a fashion consistent with orientation to upstream natal tributaries. Specifically, many stocks returning to tributaries along the south shore of the Columbia River tended to show a south shore ladder bias and vice versa. The degree of route segregation among populations increased as individual stocks neared natal tributaries, was highest when tributaries were either large (i.e., Snake River) or in close proximity to a downstream dam (i.e., John Day River), and was in some cases related to discharge. Early initiation of migration routes that reflected aggregation on natal shorelines was most prevalent for Snake River salmon, some of which exhibited orientation to the "correct" shoreline hundreds of kilometres downstream from the Snake River confluence. The observed preferences may have underestimated the onset of route selection and magnitude of ladder biases because all fish were randomly (relative to their origin) released on either the north or south shore of the Columbia River after tagging, effectively resetting any aggregation to natal shorelines that had occurred downstream of Bonneville Dam prior to tagging. The largely nonsignificant results at Bonneville Dam may reflect this release site protocol, the relative complexity of the tailrace and fishways at Bonneville Dam, or for stocks returning to tributaries of Bonneville reservoir, the relatively small size of these tributaries. Ladder use in other stocks did not display these patterns, perhaps because of small natal tributary size, long distance to the actual spawning grounds, or inadequate sample size. While inferring process from pattern can be prob-

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Table 2. Results of *G* tests comparing ladder use frequencies at dams by radio-tagged Chinook salmon (*Oncorhynchus tshawytscha*) stocks.

					Route effects (df = 1)					
			Year effects (df = 5)		50:50		Counts at ladders[a]			
Dam	Stock	*n*	*G*(h)	*P*	*G*(p)	*P*	*G*(p)	*P*	Exp %	Obs %
Bonneville	Wind	387	31.4	<0.0001	0.6	0.4463	0.1	0.7642	47.3	48.1
	Little White Salmon	225	4.0	0.5566	2.0	0.1615	10.1	0.0015	44.1	54.7
	White Salmon	34	5.7	0.3386	0.0	0.9999	0.7	0.3994	42.8	50.0
	Hood[b]	19	2.5	0.4735	4.4	0.0351	7.4	0.0064	42.8	73.7
	Klickitat	94	1.3	0.9318	0.2	0.6801	1.2	0.2774	46.5	52.1
	Deschutes	337	8.9	0.1135	1.3	0.2524	1.7	0.1910	49.6	53.1
	John Day	127	9.4	0.0927	1.8	0.1821	3.9	0.0472	47.1	55.9
	Umatilla	65	1.6	0.9049	0.8	0.3833	0.1	0.8065	46.2	44.6
	Snake	1530	16.2	0.0064	2.4	0.1253	44.1	<0.0001	43.5	52.0
	Yakima	298	11.6	0.0404	0.1	0.7290	8.0	0.0047	40.9	49.0
	Upper Columbia	1225	30.2	<0.0001	0.5	0.4751	15.7	<0.0001	43.4	49.0
The Dalles	Deschutes	327	1.8	0.8748	59.2	<0.0001	30.7	<0.0001	56.1	70.9
	John Day	119	7.9	0.1641	0.0	0.9203	0.6	0.4274	53.2	49.6
	Umatilla	63	9.9	0.0781	7.1	0.0075	5.8	0.0160	51.7	66.7
	Snake	1507	83.2	<0.0001	111.3	<0.0001	6.3	0.0121	60.4	63.5
	Yakima	292	12.1	0.0336	6.1	0.0138	1.9	0.1681	61.1	57.2
	Upper Columbia	1212	52.3	<0.0001	73.0	<0.0001	4.4	0.0364	59.3	62.2
John Day	John Day	127	8.0	0.1562	121.9	<0.0001	61.7	<0.0001	65.7	94.5
	Umatilla	64	13.9	0.0161	6.4	0.0117	0.0	0.8875	64.9	65.6
	Snake	1506	82.7	<0.0001	286.3	<0.0001	39.5	<0.0001	63.8	71.4
	Yakima	293	18.8	0.0021	0.0	0.8625	9.3	<0.0001	59.4	50.5
	Upper Columbia	1183	38.7	<0.0001	51.1	<0.0001	17.3	<0.0001	66.1	60.4
McNary	Snake	1519	89.8	<0.0001	231.2	<0.0001	98.4	<0.0001	56.9	69.3
	Yakima	298	4.5	0.4866	6.5	0.0107	20.5	<0.0001	55.7	42.6
	Upper Columbia	1225	15.1	0.0102	91.7	<0.0001	108.4	<0.0001	51.2	36.4

Note: Tests of year effects examined whether ladder use varied between years within stock. Route effect tests compared observed ladder use frequencies with frequencies based on random 50:50 passage (null hypothesis 1) and frequencies based on total counts at ladders (null hypothesis 2), including expected (Exp %) and observed (Obs %) percentages of salmon using south shore ladders.

[a]Expected percentages are based on total adult Chinook salmon counted at ladders during the date range when 90% of each radio-tagged stock passed each year.

[b]Year effects df = 3.

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Fig. 4. Mean annual Chinook salmon (*Oncorhynchus tshawytscha*) ladder selection biases (observed percentage – expected percentage, ±1 standard error) for stocks returning to the high discharge Snake and Columbia rivers (shaded bars) and all other stocks (open bars). Positive biases indicate greater than expected passage through the ladder on the same shore as the natal tributary. Expected values were generated from the total number of salmon passing each ladder during the same period. Number of stock years are noted above each bar.

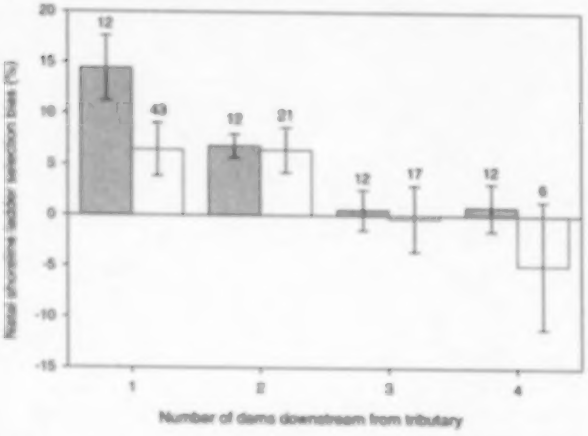
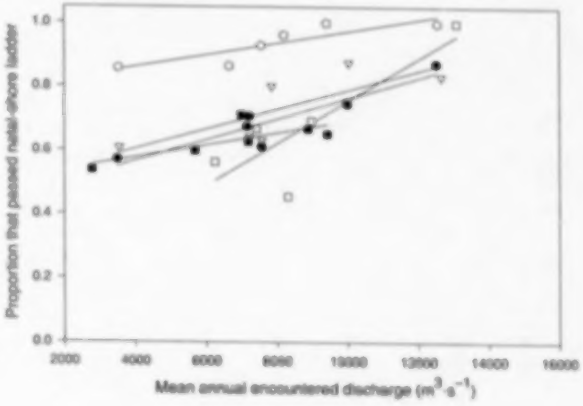


Fig. 5. Annual proportions of Chinook salmon (*Oncorhynchus tshawytscha*) stocks that selected natal shore ladder routes at dams in relation to mean river discharge each stock encountered at each dam during each run year. Plot includes only those stocks with significant ($P < 0.10$) correlations: John Day River stock at John Day Dam (○); Umatilla River stock at John Day Dam (□); Snake River stock at John Day Dam (▽); Snake River stock at McNary Dam (●); and upper Columbia River stock at McNary Dam (■).



lematic, below we use these data to speculate on potential processes affecting ladder choice by returning adults.

The physical and chemical environment of the lower Columbia River provides several potentially influential orientation cues to upstream migrants. Perhaps the most conspicuous cue is the presence of tributary plumes, which create lateral gradients in physical and chemical properties downstream from confluences. In the Columbia River, plumes from the large Snake River are often visible 175 km downstream at John Day Dam, despite the mixing that occurs at McNary Dam (Chapman et al. 1997; Scheibe and Richmond 2002). Plumes from smaller Columbia River tributaries can also be conspicuous, particularly during the seasonal snowmelt events that coincide with spring–summer Chinook salmon migrations. Measurement of plume characteristics in the Columbia River was beyond the scope of this study, but their general properties are well established (Fischer et al. 1979; Rutherford 1994). In large, low-gradient systems, complete mixing below confluences may not occur until up to several hundred river widths downstream (Rutherford 1994), and sediment, dissolved gas, and temperature plumes can remain hydrologically distinct over hundreds of kilometres (Fischer et al. 1979; Scheibe and Richmond 2002). We frequently observed salmon switching river banks after being released below Bonneville Dam (see also Johnson 2003), suggesting the potential for individuals to sample for any lateral gradients in orientation cues created by tributary plumes and to aggregate on the shoreline with the strongest cues.

A variety of orientation cues within plumes may have influenced ladder choice by adult salmon. Among these, olfactory cues probably had the strongest effect. The use of olfaction during homing by salmon has been experimentally established in fresh water using both occlusion (reviewed in Hasler and Scholz 1983) and chemical imprinting techniques (Nevitt et al. 1994; Dittman et al. 1996). Adult salmon recognize home stream odors as well as odors encountered during juvenile outmigration during a sequential imprinting process (Harden Jones 1968; Quinn et al. 1989). Interruptions to imprinting, such as displacement or transportation of juveniles, have resulted in reduced homing for both Pacific (*Oncorhynchus* spp.) and Atlantic salmon (*Salmo salar*) (Jonsson et al. 1994; Chapman et al. 1997; McCormick et al. 1998). The downstream distances that adult salmon can detect odors encountered during imprinting likely depend upon dilution levels, the duration and quality of imprinting, discharge volume, stratification, or prevailing winds or currents (reviewed in Leggett 1977). Our radiotelemetry results suggest that lateral gradients in odors or other cues significantly influenced Columbia River Chinook salmon behavior at distances that range from less than 10 km downstream of confluences for stocks returning to smaller, low-discharge tributaries to several hundred kilometres for those returning to high-discharge sites, such as the Snake River.

Salmon and many other aquatic organisms also orient to other physical and chemical gradients (Vogel 1996; Vickers 2000), such as temperature (Berman and Quinn 1991; Friedland et al. 2001), turbidity, salinity, and flow (Døving et al. 1985; Carton and Montgomery 2003). During the spring–summer Chinook salmon migration, the Columbia River is warmer than most tributaries draining the Cascade Range in western Oregon and Washington and is typically cooler than the Snake River (Quinn et al. 1997), resulting in lateral and vertical thermal gradients. The telemetry data could not be used to assess the relative importance of chemical versus nonchemical physical cues, though it is likely that they differ between spatial and temporal scales and that both attraction and aversion (i.e., avoidance of warm water) may influence fish behaviors.

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While the literature suggests olfaction is the dominant freshwater homing mechanism in salmon, particularly as fish near natal sites, additional reference systems may be important. Sun and celestial compass, geomagnetic (Montgomery and Walker 2001; Boles and Lohmann 2003), and (or) geographical landmark (route reversal) tactics (Odling-Smee and Braithwaite 2003) have been reported for a variety of fishes and other aquatic animals (reviewed in Leggett 1977). However, if adult salmon were primarily using fixed mechanisms to orient, we would expect consistent north or south shore route use regardless of distance from natal tributary or environmental conditions — a pattern not clearly supported by these data. In addition, many salmon overshoot natal tributaries by tens to hundreds of kilometres before returning downstream (Boggs et al. 2004), suggesting many adults pass natal tributaries on the wrong shore — an unlikely behavior if precise sun or compass orientation mechanisms were dominant.

The observed ladder bias by Snake River salmon as far downstream as John Day Dam and possibly as far as Bonneville Dam, compared with little evidence for ladder bias in small-tributary stocks except over short distances, was also consistent with orientation to lateral gradients because large tributaries should create longer plumes. However, irrespective of stock and natal destination, there was a strong tendency for selection of specific ladders, and these underlying preferences were related to variability in Columbia River discharge and resulting flows from dam powerhouses and spillways. As discharge increased, both within and among years, salmon were more likely to use the south shore ladder at John Day and McNary dams; salmon preferred the south shore ladder at The Dalles Dam, but increasingly used the north shore ladder as discharge increased and more water was released over the spillway near the north shore. Salmon also favored the north shore ladder at Bonneville Dam in later years, when more river water was directed through the north powerhouse (D. Clugston, US Army Corps of Engineers, Box 2946, Portland, OR 97208, USA, personal communication). This suggests that rheotaxis was a key orientation component during migration, with fish favoring flow pathways at dams with greater attraction volume and (or) velocity. This behavior could either reduce or reinforce the use of natal shoreline routes at dams, depending on the destination of individual fish and the relative attractiveness of flow near the individual's natal shoreline. For example, the preference for south shore ladders by Snake River salmon at John Day and McNary dams may have been related to both the presence of olfactory homing cues within the Snake River plume and the distribution of attractive flows at these dams. Managers seeking to improve fish passage efficiency should consider strategies that promote the retention of plume structures and limit operations that result in cross-channel mixing.

Overall these results suggest that strong swimmers like salmon almost certainly integrate rheotactic and olfactory strategies during homing migration. Odor-conditioned rheotaxis, or the reliance on flow as an orienting stimulus in combination with odor detection (Weissburg 2000), is generally consistent with our results and more specifically with the migration control model developed by Johnsen (1982) and modified by Quinn (2005). The control model hypothesizes that salmonids use rheotaxis and odor recognition to navigate through river networks, using positive (straight upstream) rheotaxis in the presence of familiar or home stream odors, lateral searching or upstream zigzagging along tributary plume boundaries when cues are mixed, or downstream retreat when odors are absent (Johnsen 1982; Quinn 2005). These behaviors may balance the costs and benefits of efficient homing with the energetic constraints imposed by moving through a complex flow environment. Such tradeoffs to maximize energetically efficient movement appear to be used by many other genera in diverse aquatic habitats (Zimmer-Faust et al. 1995; Baker et al. 2002). In addition, conditioned rheotaxis may explain the widespread tendency of salmon to follow nearshore routes in large river systems (e.g., Daum and Osborne 1998; Hinch et al. 2002), including the Columbia River (Johnson 2003; Reischel and Bjornn 2003), though hydrodynamic factors also appear to play a strong role (Hughes 2004).

A potential cost of conditioned rheotaxis is that fish that infrequently sample cues along both shorelines may be more likely to miss natal confluences, and this effect may be amplified in reservoir environments. Such overshooting can result in fallback at upstream dams (Boggs et al. 2004) as fish return downstream in search of natal sites. Downstream movements have important implications in regulated rivers, as fallback at dams reduces both effective migration speeds (Keefer et al. 2004*a*) and survival (Keefer et al. 2005).

Predictable salmon orientation to tributary plumes or to specific shorelines or fishways also has important implications for harvest management, particularly in large rivers with multiple stocks. For example, mixed-stock fisheries near the south shore of the Columbia River downstream from John Day Dam may disproportionately harvest John Day River salmon or even Snake River salmon. Given the evidence for long-distance orientation, shoreline-specific fishing regulations, particularly near confluences, could reduce impacts on stocks of conservation concern in mixed-stock fisheries.

In summary, the observed behaviors suggest that salmon homing in large rivers use a hierarchy of orientation mechanisms. The relative importance of orientation to lateral gradients created by tributary plumes appears to be related to tributary size and proximity, river discharge, and dam operations that affect the distribution of flow. Salmon route selection patterns reported here were consistent with predictions of the classic salmon-homing studies (e.g., Johnsen and Hasler 1980; Hasler and Scholz 1983), where returning adults used olfaction to orient to experimentally manipulated odor plumes in small streams. Whereas the experimental studies described homing strategies on the scale of hundreds of metres, the current results indicate similar phenomena may occur over tens to hundreds of kilometres, a much broader scale than has been described for most other aquatic taxa. We note that this study occurred in an environment transformed by hydroelectric development and that behaviors may be different in unregulated rivers. Tributary plumes may persist for longer distances in reservoirs than in free-flowing rivers, because deeper water columns and slower water velocities in reservoirs can lower vertical and horizontal mixing rates. Such changes may facilitate fish navigation. In contrast, dams produce turbulent mixing and alter flow path-

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ways, providing potentially confusing migration cues; therefore, the net effect of dams relative to historical conditions remains unknown. Although the study system is highly altered, these salmon route-selection results offer compelling examples of fish orientation in a large and complex riverine environment and suggest the potential for fish orientation to large-scale plumes in lacustrine, estuarine, coastal shelf, and pelagic waters.

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Distribution of biofilm exopolymeric substances in littoral sediments of Canadian Shield lakes: the effects of light and substrate

Hélène Cyr and Kathryn E. Morton

Abstract: Benthic algal and microbial communities release organic compounds that perform many important functions in food webs and nutrient dynamics. In this study we compared the content and composition of extracellular substances from different types of sediments in the shallow littoral areas of clear and dark Canadian Shield lakes. We report high content of extracellular substances in these littoral sediments, in terms of both carbohydrates and proteins. Total carbohydrate contents increased with increasing sediment chlorophyll content in a relationship very similar to that reported for marine intertidal mudflats. Light availability and sediment type affected the content and composition of exopolymeric substances (EPS) in complex ways. More loosely bound (colloidal) EPS were measured in sandy sediments of dark lakes. Colloidal EPS content did not vary with changes in sediment particle size in clear lakes but may have been positively related to sediment particle size in dark lakes. Tightly bound (capsular) EPS increased with sediment water content with no effect of light availability. These relationships are consistent across the seven lakes. The role of these carbohydrates and proteins in the productivity and nutrient dynamics of littoral areas in lakes remains to be tested.

Résumé : Les communautés benthiques d'algues et de microorganismes rejettent des composés organiques qui jouent des rôles importants dans les réseaux alimentaires et la dynamique des nutriments. Nous comparons dans cette étude la quantité et la composition des substances extracellulaires dans différents types de sédiments dans la zone littorale peu profonde de lacs clairs et foncés du Bouclier canadien. Il y a un fort contenu de substances extracellulaires dans ces sédiments littoraux, tant des hydrates de carbone que des protéines. Le contenu total en hydrates de carbone augmente en fonction de la teneur en chlorophylle des sédiments, selon une relation semblable à celle signalée dans les platières boueuses intertidales. La disponibilité de la lumière et le type de sédiments affectent de manière complexe la quantité et la composition des substances exopolymères (« EPS »). On peut mesurer des EPS à liens plus lâches (colloïdales) dans les sédiments sablonneux des lacs foncés. Le contenu en EPS colloïdales ne varie pas en fonction des changements dans la taille des particules de sédiments dans les lacs clairs, mais peut être en corrélation positive avec la taille des particules de sédiments dans les lacs foncés. Il y a une augmentation des EPS à liens serrés (capsulaires) en fonction du contenu hydrique des sédiments; la disponibilité de la lumière reste cependant sans effet. Ces relations sont semblables dans les sept lacs. Les rôles de ces hydrates de carbone et ces protéines dans la productivité et la dynamique des nutriments dans les zones littorales des lacs restent à vérifier.

[Traduit par la Rédaction]

Introduction

A large proportion of the productivity of algae, bacteria, and macrophytes in freshwater and marine ecosystems is released extracellularly. These organic compounds represent, on average, 1%–10% of net primary production in macrophytes (Brylinsky 1977; Sorrell et al. 2001), 10%–20% in planktonic communities (Baines and Pace 1991; Morán et al. 2002), and at least double this proportion (on average 30%–40%) in benthic algal communities (Goto et al. 1999; Underwood and Paterson 2003). The organic compounds released by benthic algae and bacteria contain (exo)polymeric substances (EPS), which have received much attention because of the important role they play in many ecosystems (e.g., marine intertidal flats, streams, deserts; Mazor et al. 1996; Decho 2000; Battin et al. 2003). For example, EPS have been shown to stabilize sediments and soils, modify nutrient exchanges at the sediment–water interface, affect the breakdown of organic matter, provide nutritious sources of food for invertebrates, and enhance the transfer of contaminants through food webs (Decho 2000; Fischer 2003). High EPS content was recently reported from the shallow littoral zone of a Canadian Shield lake (Hirst et al. 2003), suggesting that EPS could be an important component of benthic foodwebs

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in oligotrophic lakes. Algae and cyanobacteria can be important producers of extracellular substances. This has been demonstrated in laboratory monocultures (e.g., Hoagand et al. 1993; Otero and Vincenzini 2003; Underwood and Paterson 2003) and in natural ecosystems (e.g., Underwood and Smith 1998). The carbohydrate content in marine intertidal mudflats ranges over 2–3 orders of magnitude (across time and space) and is significantly related to chlorophyll *a*, a measure of algal biomass (Underwood and Smith 1998; van Duyl et al. 1999; de Brouwer et al. 2003). It is unknown whether these models apply to sediments in the shallow littoral areas of lakes.

The release of large EPS by algae is affected by light and nutrient availability. Several studies show that the production of EPS carbohydrates is very tightly coupled to photosynthesis and increases with increasing light availability (e.g., Staats et al. 2000*b*). However, per unit production, more EPS are released when algal growth is limited by light or nutrients (Maranon et al. 2004; Otero and Vincenzini 2004; Underwood et al. 2004). Light availability decreases exponentially with increasing depth but also varies with lake transparency. In this study, we compare sediments collected at the same depth in seven lakes and focus on the potential effect of lake transparency on sediment EPS content. We also compare a wide range of sediments with different nutrient contents.

Bacteria can also produce copious amounts of EPS, which contain carbohydrates and are sometimes rich in proteins and lipids (Flemming and Wingender 2001). The amount and chemical composition of EPS released by bacteria vary among strains but are also greatly affected by environmental conditions (Decho 1990; Liu and Fang 2003). These relationships have been studied extensively in wastewater treatment, where engineers are interested in maximizing the settling properties of activated sludge. They have clearly shown that the chemical composition of the EPS (specifically their protein to carbohydrate ratio) determines how cohesive they are and how tightly they bind to surfaces (Liu and Fang 2003). This should, in turn, determine their role in the environment. Bacteria are very abundant and active in sediments (Haglund et al. 2002), and their productivity increases with the organic content of the sediments (Sander and Kalff 1993). Bacteria have been shown to quickly break down labile carbohydrates (Fischer 2003; Kirchman 2003; Decho et al. 2005), but their role in the production of EPS in natural sediments is unknown.

A few studies have suggested that the EPS content of sediments could be predicted from sediment water content, a simple measure of sediment texture (e.g., Paterson et al. 2000; Yallop et al. 2000). Hirst et al. (2003) also reported relationships between EPS and porewater content in the shallow sediments of a polymictic lake. Because there have been so few studies in freshwater on soft substrates, it is unknown whether these results can be generalized to other lakes. Sediment water content is very simple to measure and could provide a useful predictor of the EPS content in soft littoral sediments, especially given the notorious heterogeneity of substrates found in the shallow areas of lakes.

In this study we compare the content and composition of EPS in the littoral sediments of seven Canadian Shield lakes to address three main questions. First, are the high EPS content and the strong relationships between EPS content and sediment porewater content reported by Hirst et al. (2003) for a shallow polymictic basin also found in other Canadian Shield lakes? Second, is the relationship found in marine intertidal mudflats between sediment carbohydrate content and algal biomass (Underwood and Smith 1998) applicable to lakes? Third, is the amount and composition of EPS produced in situ by complex natural communities of algae, bacteria, and fungi related to environmental conditions, specifically light availability, nutrients, and sediment type? Our goal is to develop models that can be used to predict the amount and composition of EPS in lake sediments, given the notorious heterogeneity of littoral areas.

Materials and methods

Sampling sites

We sampled seven lakes on the Canadian Precambrian Shield in the Dorset and Algonquin Park areas, Ontario, Canada (Table 1). These lakes were chosen to cover a range of productivity (oligotrophic to oligo-mesotrophic, based on epilimnetic total phosphorus (TP) concentration), which in this region is largely determined by the size of watersheds and the distribution of glacial till deposits (Dillon and Molot 1996). We expected this gradient of lake productivity to provide a gradient in the nutrient content of littoral sediments, but epilimnetic TP concentration in these lakes is poorly related to the TP content of littoral sediments (Table 1). Nevertheless, sediment TP content ranged over an order of magnitude between our sampling sites (Table 1). We also selected clear and dark lakes to test the effect of light availability. Lake transparency in this region varies with the concentration of colored dissolved organic carbon (DOC) in the water column, which is only weakly related to total DOC (Molot and Dillon 1997*a*). The lakes we selected covered a modest range of epilimnetic DOC concentration (2.2–7.3 mg·L^{-1}), but all low DOC lakes (2.2–2.4 mg·L^{-1}) were clear and most high DOC lakes (>4 mg·L^{-1}) were darker (Table 1). The only exception was Annie Bay (Lake Opeongo), a large and relatively clear lake basin with high DOC (4.1 mg·L^{-1}). The photooxidative loss of coloured DOC is well documented in large lake basins with high water retention times (Molot and Dillon 1997*a*, 1997*b*).

The lakes have been impacted by different human activities. Lakes Opeongo and Costello are located in a provincial park, but their catchment was logged in the later part of the 19th century, and water levels were raised to facilitate the transport of logs. Additionally, St. Jacques et al. (2005) provides paleoecological evidence of increased productivity in Lake Opeongo starting in the 1960s. Increased nutrient inputs have affected several lakes in the Dorset area, where cottages and recreational facilities have been developing rapidly (e.g., Harp and Paint lakes; Dillon and Molot 1996). Moreover, all lakes in this region have been exposed to acid precipitations, which have affected some lakes more than others (e.g., Plastic Lake; Jefferies et al. 2003).

All sampling sites were located in the shallow littoral zone (2–2.4 m depth). Within each lake, 4–6 sampling sites were distributed around the basin at wind-exposed and more protected sites to maximize the range of sediment types sampled (from coarse sand to mud). All sites were located around the edge of the main basins (i.e., not in small embayments).

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Table 1. Lake characteristics and information on sampling sites.

Lake	Location	Surface area (ha)	Maximum lake depth (m)	Mean lake depth (m)	Epilimnion TP (μg·L^{-1})	Epilimnion DOC (mg·L^{-1})	Sampling dates (2001)	Number of sites sampled	Mean sampling depth (m)	Sediment TP (μg·g^{-1} dm)	Bottom light ($\%\ I_0$)
Solitaire	45°22′N, 79°08′W	124	31	13.3	5.3[a]	2.2[a]	26 July	5	2.1	75–135	55
Harp	45°23′N, 79°08′W	71	38	13.3	5.4[a]	4.0[a]	11 July	4	2.2	150–510	18
Opeongo, Annie Bay	45°43′N, 78°17′W	440	24	9.6	7.7[b]	4.1[c]	3 Aug.	6	2	210–490	40
Halls	45°06′N, 78°44′W	396	35	10.4	5.2[a]	2.4[a]	9–10 July	5	2.1	250–750	47
Plastic	45°11′N, 78°50′W	32	16	7.9	4.1[a]	2.2[a]	25 July	6	2.4	360–640	49
Costello	45°36′N, 78°20′W	39	19	11.7	9.8[a]	4.9[a]	4 Aug.	4	2.2	380–600	15
Paint	45°13′N, 78°57′W	145	21	6.9	8.2[a]	7.3[a]	12–14 June	6	2.1	560–845	18

Note: Lakes are ordered by increasing sediment total phosphorus (TP) content. Sediment TP shows the range in site means. Bottom light is the percentage of surface irradiance (I_0) that penetrates to the bottom, based on mean sampling depth. DOC, dissolved organic carbon; dm, dry mass.

[a]A. Paterson, Ontario Ministry of the Environment, Dorset Research Centre, Dorset, Ontario. Costello: $n = 1$ in 2002 for TP, $n = 1$ in 1982 for DOC; Halls: $n = 2$ in 2002–2003 for TP, $n = 1$ in 1990 for DOC; Harp and Plastic: ice-free season average from 2001; Paint: $n = 3$ in 2002–2003 for TP, $n = 1$ in 2002 for DOC; Solitaire: spring–fall 1998–2003, $n = 6$ for DOC, $n = 5$ for TP.

[b]G. Sprules and H. Cyr, Department of Zoology, University of Toronto, Toronto, Ontario, summer 2000.

[c]A. Blukacz and G. Sprules, Department of Zoology, University of Toronto at Mississauga, Mississauga, Ontario, $n = 5$, July–October 2001.

Most sites had sparse or no vegetation cover, with the exception of three sites located in beds of *Isoetes* (Annie Bay site 1, Halls Lake site 4) and of *Juncus* (Paint Lake site 5). The EPS data from these sites did not stand out and were kept in the analyses.

Sediment sampling

At each sampling site, 5–6 sediment cores were collected 3 m apart along a transect running parallel to shore. A diver cored the sediments by hand using a 5.6 cm diameter lexan corer. Each intact core was carefully brought back to the boat, where a piston was used to extrude and slice the surface 1 cm of sediments. All samples were kept in a dark cooler until return to our field laboratory. Sediment samples were then thoroughly stirred by hand and 2.5–3 g aliquots were frozen in liquid nitrogen for EPS and pigment analyses. The samples were later stored in a –80 °C freezer until analysis.

Sediment analyses

The rest of the sediments from each combined surface sample was used to measure bulk density, porewater content, organic matter content, total nutrient content, and mean particle size of the sediments. The sediments were first weighed to calculate bulk density (in g·cm^{-2}). The sediments were then homogenized, and two replicate subsamples were dried at 60 °C for 2 days and reweighed to determine porewater content (as percent of sediment wet mass). The dried sediments were ground and homogenized, and a subsample was used for nitrogen (TN) and carbon analysis on a CHN analyser (CHN-600; LECO Co., St. Joseph, Michigan, USA). The remaining sediments were ashed at 550 °C for 2 h to determine organic matter content (loss on ignition, as percent of sediment dry mass). The ashed sediments were then digested in 1 mol·L^{-1} HCl, and TP concentration was determined colorimetrically using the ascorbic acid reduction method (Andersen 1976).

The remaining sediments in each sample were used to measure sediment particle sizes. Each sample was size fractionated by wet-sieving through Wentworth grade sieves (8, 4, 2, 1, 0.5, 0.25, 0.125, 0.063, and 0.035 mm mesh size). The sediments retained on each sieve were dried at 60 °C for 24 h and weighed. Silt finer than 0.035 mm was left to settle for 3 weeks, dried at 60 °C, and weighed. These fine sediments were assigned to the next smaller size fraction (0.017 mm). The geometric mean particle size of each sediment sample was calculated as a mean phi value (φ; Buchanan 1984):

$$\varphi = \sum_i (-\log_2(S_i)\mathrm{DM}_i) / \sum_i (\mathrm{DM}_i)$$

where S_i is the mesh size (in mm) on which each size fraction i is retained and DM_i is the dry mass of sediment particles in size fraction i (g). Mean phi values increase as the sediments get finer.

Extraction of colloidal and capsular exopolymers

We measured two fractions of exopolymers, which we will call colloidal and capsular to follow the terminology used by Underwood et al. (1995). The colloidal fraction includes carbohydrates and proteins that are loosely bound to sediment particles, bacteria, and algal cells. The capsular

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fraction contains tightly bound carbohydrates and proteins that require a lengthier extraction using ethylenediaminetetraacetic acid (EDTA; Fig. 1).

The sediments were defrosted in an ice bath, and the colloidal fraction was extracted by adding 10 mL of deionized water to 2–3 g (fresh mass) of sediment and mixing at room temperature on a reciprocating shaker table for 20 min. The sample was then centrifuged at 23 000*g* for 30 min in a refrigerated (4 °C) superspeed centrifuge (Sorval model RC-5B; Ivan Sorval Inc., Norwalk, Connecticut). The supernatant was decanted, mixed with cold ethanol to a final concentration of 60%, and stored overnight at –10 °C to precipitate the high molecular mass exopolymeric substances (Decho 1990). This mixture was then centrifuged at 23 000*g* for 10 min and the supernatant, which contains monomers and small polymers (de Brouwer and Stal 2001), was decanted and analysed for proteins and carbohydrates (see methods below). The precipitate, which contains polymeric substances, was redissolved in 1 mL of deionized water and triplicate subsamples were analysed for proteins. An additional 1 mL of deionized water was then added to the remaining sample and triplicate subsamples were analysed for carbohydrates.

The capsular fraction was extracted with EDTA. As recommended by Platt et al. (1985), we compared the efficiency of different concentrations of EDTA in extracting capsular EPS from freshwater sediments. The concentration of proteins measured increased as the concentration of EDTA was raised from 1 to 5 mmol·L^{-1} and remained relatively constant between 5 and 20 mmol·L^{-1} EDTA (Fig. 2). This suggests that an EDTA solution < 5 mmol·L^{-1} is insufficient to completely extract the capsular proteins from these sediments. However, raising EDTA concentrations beyond 20 mmol·L^{-1} resulted in a sharp increase in proteins (Fig. 2), which is likely due to cell damage and leakage of cellular content. Based on these results, we selected a 15 mmol·L^{-1} EDTA solution for our analyses. Platt et al. (1985) reported negligible contamination from intracellular polymeric material (only 3% of total glucose-6-phosphate dehydrogenase recovered) when using a slightly weaker (10 mmol·L^{-1}) EDTA solution with freshwater sediments. The concentration of EDTA we used is 10 times lower than what is used with marine sediments, where intracellular contamination is not a problem (Underwood et al. 1995), and three times lower than what has been used with stream sediments (Battin and Sengschmitt 1999).

The capsular fraction was extracted by adding 10 mL of 15 mmol·L^{-1} EDTA to the sediment pellet (modified from Platt et al. 1985), mixing thoroughly, and incubating at room temperature on a reciprocating shaker table for 4 h (Underwood et al. 1995). The samples were centrifuged at 23 000*g* for 20 min. The high molecular mass exopolymers in the supernatant were precipitated in cold ethanol, as described above. The mixture was then centrifuged at 23 000*g* for 10 min. Carbohydrates and proteins were measured in the supernatant (monomers and small polymers) and in the precipitate (large polymers), as described for the colloidal fraction.

Measurement of carbohydrates and proteins

Carbohydrates were measured using the phenol – sulfuric acid method (Dubois et al. 1956), as modified by Liu et al. (1973) for lake sediments. We added 400 µL of aqueous phenol (75 mg·mL^{-1}) to 400 µL of sample from the colloidal or the capsular fraction and quickly added 2 mL of concentrated sulfuric acid while vortexing. Preliminary tests showed that a phenol concentration of 75 mg·mL^{-1} gave the best colour development. Colour was allowed to develop for 1 h, and then absorbances were measured at 485 nm on a spectrophotometer (Milton Roy Spectronic 1001 Plus; Milton Roy, Spectronic Instrument, Inc., Rochester, N.Y.). The absorbance values were calibrated with a glucose standard (Sigma Chemical Co., St. Louis, Mo.). Carbohydrate concentrations are reported in µg glucose equivalents (equiv.)·g^{-1} of sediment fresh mass, which were then divided by (1 – (%WC/100)), where %WC is sediment percent water content, to convert them to µg glucose equiv.·g^{-1} dry mass.

Proteins were measured with the bicinchoninic acid method (Smith et al. 1985). This method is very similar to the commonly used Lowry method, but it uses a more efficient detection reagent. Bicinchoninic acid is more sensitive, chemically more stable, and less susceptible to interferences (e.g., to EDTA) than the Folin–Ciocalteau reagent used in the Lowry method (Smith et al. 1985). We combined a 100 µL sample from either the colloidal or the capsular fraction with 2 mL of mixed reagent (BCA-1 kit; Sigma Chemical Co.), and this mixture was incubated for 30 min at 60 °C in a gently shaking heated water bath. The samples were allowed to cool to room temperature and absorbance was read at 562 nm. The absorbances were calibrated with a BSA standard (Sigma Chemical Co.). Protein concentrations are reported in µg BSA equiv.·g^{-1} of sediment fresh mass, which were then divided by (1 – (%WC/100)) to convert them to µg BSA equiv.·g^{-1} dry mass.

Pigment analyses

The pigments in two to three cores per site were measured by P.R. Leavitt (Department of Biology, University of Regina, Regina, SK S4S 0A2). The sediments were lyophilized, and the pigments were extracted with an acetone–methanol mixture and measured by reversed-phase high performance liquid chromatography (HPLC; Leavitt and Carpenter 1989). Chlorophyll *a* content is expressed in µg·g^{-1} dry mass (dm) and multiplied by sediment bulk density (in g dm·cm^{-3}) to convert it to µg·cm^{-2}. We use the molar ratio of echinenone to fucoxanthin as an indicator of the importance of cyanobacteria relative to diatoms and the ratio of chlorophyll *b* to fucoxanthin as an indicator of the importance of green algae relative to diatoms. Lugol-preserved samples were examined to confirm differences in the algal composition suggested by the pigment data.

Statistical analyses

The relationship between chlorophyll content and sediment water content was tested using regression analysis (Zar 1984) with the following model:

$$\log_{10}(\text{CHLA}) = a + b\,\text{WC}$$

where CHLA is chlorophyll *a* content, a measure of algal biomass (in µg·g^{-1} dm or µg·cm^{-2}), WC is arcsine-transformed sediment water content (arcsine(sqrt(WC))), and *a* and *b* are fitted coefficients.

The relationship between total carbohydrate content and chlorophyll content proposed by Underwood and Smith (1998)

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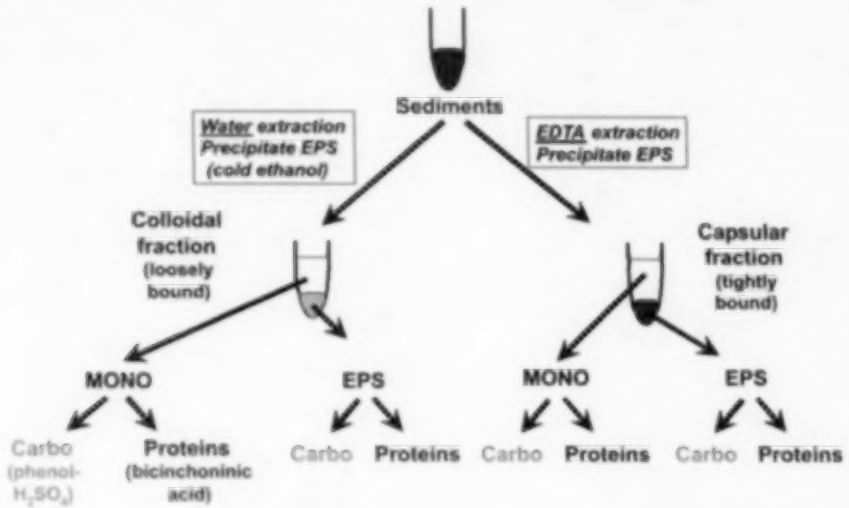
Fig. 1. Schematics of steps for the extraction of all fractions of carbohydrates and proteins from lake sediments. EDTA, ethylenediaminetetraacetic acid; MONO, monomeric and small polymeric substances; EPS, large polymeric substances; Carbo, carbohydrates.



and the effect of sediment particle size and light availability on this relationship were tested by analysis of covariance (ANCOVA) using multiple regression (Zar 1984):

$$\log_{10}(\mathrm{TCARB}_i) = a + b\log_{10}(\mathrm{CHLA}) + c\mathrm{PHI}_{<2\mathrm{mm}} + dI + e[\log_{10}(\mathrm{CHLA}) \cdot \mathrm{PHI}_{<2\mathrm{mm}}] + f[\log_{10}(\mathrm{CHLA}) \cdot I] + g[\mathrm{PHI}_{<2\mathrm{mm}} \cdot I] + h[\log_{10}(\mathrm{CHLA}) \cdot \mathrm{PHI}_{<2\mathrm{mm}} \cdot I]$$

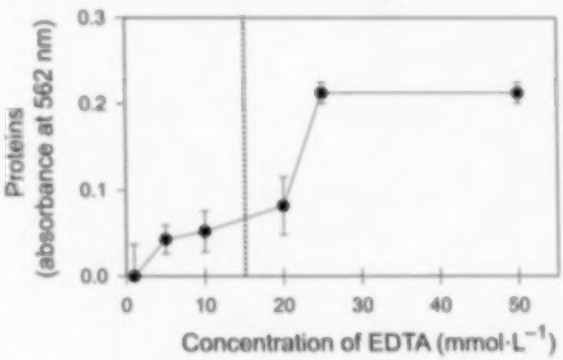
where TCARB_i is total carbohydrate content in sediments (monomers and polymers, in µg glucose equiv.·g^{-1} dm) in the colloidal and capsular fractions i, CHLA is sediment chlorophyll a content (in µg·g^{-1} dm), $\mathrm{PHI}_{<2\mathrm{mm}}$ is mean phi value, a measure of mean sediment particle size, I is a dummy variable coding for lakes with low (<20% surface irradiance; $I = 0$) or high (≥40% surface irradiance; $I = 1$) light penetration, and a–h are fitted coefficients.

We also tested two types of relationships for each EPS fraction (colloidal EPS carbohydrates, colloidal EPS proteins, capsular EPS carbohydrate, capsular EPS proteins) using multiple regression analysis. First, the relationships between each EPS fraction and sediment texture, nutrient content, and light availability were tested with the following model:

$$\log_{10}(\mathrm{EPS}_{ij}) = a + b\mathrm{PHI}_{<2\mathrm{mm}} + c\log_{10}(\mathrm{TP}) + dI + e[\mathrm{PHI}_{<2\mathrm{mm}} \cdot \log_{10}(\mathrm{TP})] + f[\mathrm{PHI}_{<2\mathrm{mm}} \cdot I] + g[\log_{10}(\mathrm{TP}) \cdot I] + h[\mathrm{PHI}_{<2\mathrm{mm}} \cdot \log_{10}(\mathrm{TP}) \cdot I]$$

where EPS_{ij} is EPS fraction i for colloidal or capsular fractions and j for carbohydrates or proteins (in µg glucose or BSA equiv.·g^{-1} fresh mass (fm)), $\mathrm{PHI}_{<2\mathrm{mm}}$ is mean phi value on <2 mm sediments, TP is sediment phosphorus content (in µg·g^{-1} dm), I is a dummy variable coding for lakes with low (<20% surface irradiance; $I = 0$) or high ($I = 1$) light penetration, and a–h are fitted coefficients for the main factors and all interaction terms.

Fig. 2. Effect of ethylenediaminetetraacetic acid (EDTA) concentration on the recovery of proteins from freshwater littoral sediments. The broken line indicates the concentration of EDTA (15 mmol·L^{-1}) that we decided to use to separate the colloidal (weakly bound) and capsular (tightly bound) fractions in our analyses.



Second, the relationships between each EPS fraction and sediment water content (a simple measure of sediment characteristics) and light availability were tested with the following model:

$$\log_{10}(\mathrm{EPS}_i) = a + b\mathrm{WC} + cI + d[\mathrm{WC} \cdot I]$$

where a–d are fitted coefficients.

All analyses were performed in Statistica for microcomputers (version 5; StatSoft Inc. 1997).

Results

We sampled a wide range of soft substrates in each lake from muds to sands to mixtures of sand and pebbles (e.g., Figs. 3*a*–3*c*,, respectively). The water content of these littoral sediments was not related to mean sediment particle size (mean phi values; Fig. 4*a*). Sediment water content was ex-

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Fig. 3. Distribution of sediment particle size in cores from (*a*) Paint Lake (PNT4-1: site 4, core 1, mud), (*b*) Annie Bay (AB5-1: site 5, core 1, sand), and (*c*) Lake Costello (COS4-5: site 4, core 5, sand with pebbles) to show the range of sediments we sampled. The vertical broken line separates the pebbles and rocks retained on sieves coarser than 2 mm mesh size from the finer material (<2 mm) used to calculate mean phi values. $Phi_{<2mm}$ is 2.8 in PNT4-1, 1.64 in AB5-1, and 1.65 in COS4-5.

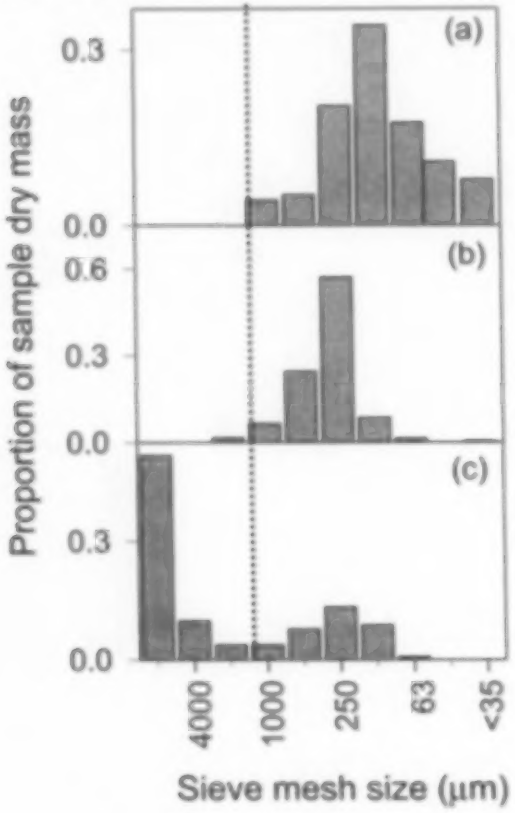
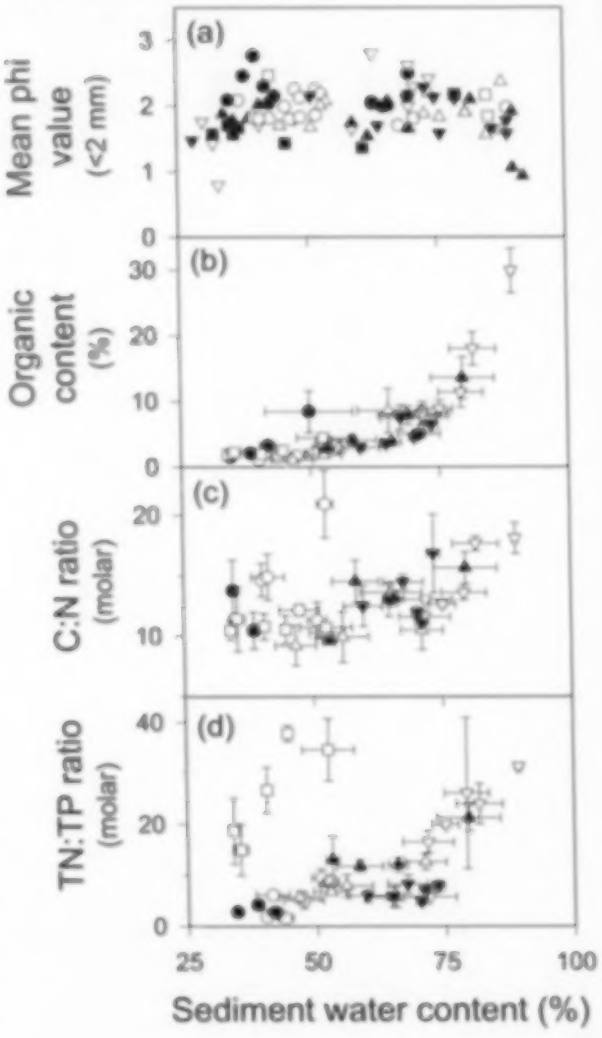


Fig. 4. Relationships between sediment water content and (*a*) sediment particle size (mean phi value), (*b*) organic content, (*c*) C:N molar ratio, and (*d*) total nitrogen to total phosphorus (TN:TP) molar ratio in the shallow littoral zone of seven Canadian Shield lakes. (*a*) Each point represents a single core. (*b–d*) The points are means for each site (± standard error), based on 4–6 cores collected ~3 m apart. The error bars represent spatial variability in the littoral zone, not analytical variability. Annie Bay (○), Costello (●), Halls (△), Harp (▲), Plastic (▽), Paint (▼), Solitaire (□).



ponentially related to the organic content (or loss on ignition (LOI)) of the sediments (Fig. 4*b*) and to the nitrogen content of sediments (not shown), which was itself very tightly related to their organic content (linear regression, $P < 0.0001$, $r^2 = 0.89$, not shown). The C:N ratio of these littoral sediments ranged from 10 to 20 (median = 13) and increased nonlinearly with increasing sediment water content (Fig. 4*c*), with the exception of three sites in Annie Bay (sites 4, 5, 6; open circles in Fig. 4*c*). At two of these Annie Bay sites, a thin layer of sediments covered the underlying rock surface. In the littoral zone, the most fluid sediments are highly organic, have high N content, and tend to have the highest C:N ratios.

The total phosphorus content of sediments varied by more than an order of magnitude among the lakes we sampled (75–845 $\mu g \cdot g^{-1}$ dm; Table 1). Two lakes clearly had low phosphorus compared with their nitrogen content (i.e., high TN:TP ratio in Fig. 4*d*). The sediments (mostly sands) in Lake Solitaire had extremely low TP content, whereas the sediments in Plastic Lake were very organic but had relatively low phosphorus compared with the nitrogen. This provides a wide range of TN:TP ratios (Fig. 4*d*) to test the effect of sediment chemical composition on the content and composition of EPS in littoral sediments.

Benthic algal communities

The biomass of benthic algae, measured as chlorophyll *a* content, was closely related to sediment water content (Fig. 5*a*) but was not related to sediment particle size (regression with

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Fig. 5. Relationship between sediment water content and chlorophyll *a* expressed (*a*) per gram dry mass (dm) of sediments and (*b*) per unit surface area. Each point is a site mean (among-core variability is not shown for clarity). Solid line: log(CHLA) = −0.6 + 0.027arcsinet(sqrt(WC)), $P < 0.0001$, $r^2 = 0.81$, $n = 34$. Annie Bay (○), Costello (●), Halls (△), Harp (▲), Plastic (▽), Paint (▼), Solitaire (□).

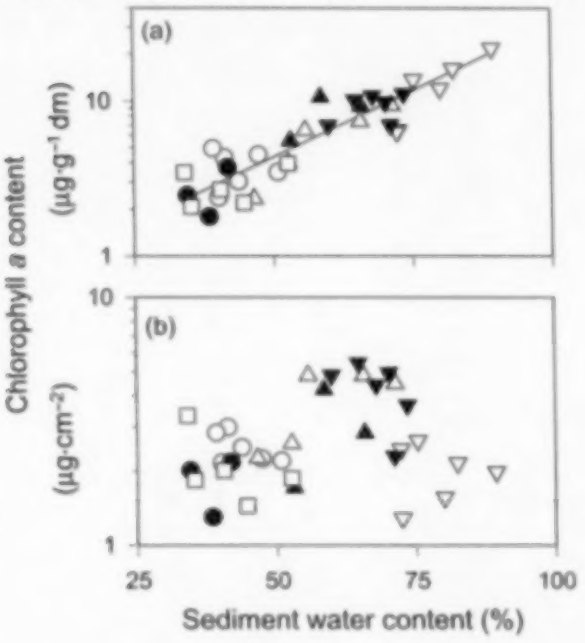
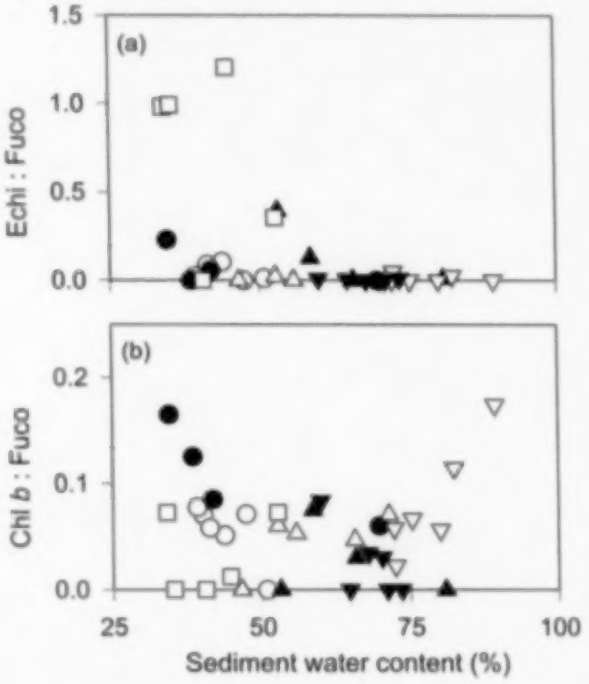


Fig. 6. Relationship between sediment water content and diagnostic pigment molar ratios (Echi, echinenone; Chl *b*, chlorophyll *b*; Fuco, fucoxanthin). (*a*) Ratios of echinenone to fucoxanthin indicate the importance of cyanobacteria relative to diatoms, (*b*) ratios of chlorophyll *b* to fucoxanthin indicate the importance of green algae relative to diatoms. Each point is a site mean (among-core variability is not shown for clarity). Annie Bay (○), Costello (●), Halls (△), Harp (▲), Plastic (▽), Paint (▼), Solitaire (□).



mean phi value, $P > 0.6$; not shown). When expressed per unit area, however, chlorophyll *a* content varied only fourfold among all littoral sites (1.3–5.5 µg chlorophyll *a*·cm^{-2}) and was not related to sediment water content (regression, $P = 0.3$; Fig. 5*b*) and only weakly related to sediment particle size (regression with mean phi value, $r^2 = 0.14$, $P = 0.04$; not shown). Areal chlorophyll *a* was highest in sediments with intermediate water content (60%–75%; Fig. 5*b*) and had a weak tendency to increase in finer sediments.

Most benthic algal communities were dominated by diatoms. This was suggested by the pigment analysis and confirmed by qualitative microscopic observations. A few sites also had a relatively large representation of cyanobacteria or green algae (Fig. 6). The pigment analysis showed high echinenone–fucoxanthin ratio at most sites in Lake Solitaire (Fig. 6*a*), suggesting the importance of cyanobacteria in the littoral sediments of this lake. Microscopic observations confirmed the presence of filamentous and colonial cyanobacteria (e.g., *Komvophoron*, *Nostoc*, *Oscillatoria*), either loosely associated or tightly attached to sand grains. Echinenone was also measured at several sites with low water content (<60%) in Harp Lake, Lake Costello, and Annie Bay. Echinenone was not detected or only found at very low concentrations in fluid and highly organic muds (sediments with >70% water content; Fig. 6*a*). Cyanobacteria were present at these sites (H. Cyr, personal observation) but were less prevalent.

Chlorophyll *b*, the diagnostic pigment for green algae, was detected at many sites, but its concentration was generally low compared with that of fucoxanthin (Fig. 6*b*). The highest chlorophyll *b* – fucoxanthin ratios were measured in the sediments with the lowest water content in Lake Costello and with high water content in Plastic Lake sediments (Fig. 6*b*). The green algae in Lake Costello were mostly *Gloeocystis* and a filamentous green alga. Desmids were very abundant in Plastic Lake. Filamentous and colonial green algae were also present in other lakes but were not abundant.

Algal biomass and extracellular carbohydrates

The total carbohydrate content (monomers and polymers) in littoral sediments ranged an order of magnitude between sampling sites (Table 2) and was closely related to chlorophyll *a* content, an indicator of algal biomass (Fig. 7). The total carbohydrate content (TCARB$_t$) was positively related to sediment chlorophyll *a* content (CHLA), both for the loosely bound colloidal fraction ($\log_{10}\text{TCARB}_{coll} = 2.3 + 0.53\log_{10}(\text{CHLA}) - 0.18\text{PHI}_{<2mm}$; regression, $P < 0.0001$, $r^2 = 0.63$, $n = 28$; Fig. 7*a*) and the more tightly bound capsular fraction ($\log_{10}\text{TCARB}_{caps} = 1.72 + 0.89\log_{10}(\text{CHLA})$; regression, $P < 0.0001$, $r^2 = 0.82$, $n = 27$; Fig. 7*b*). Finer sediments (with high $\text{PHI}_{<2mm}$) had less colloidal carbohydrates (main $\text{PHI}_{<2mm}$ effect, $P = 0.02$), but there was no sig-

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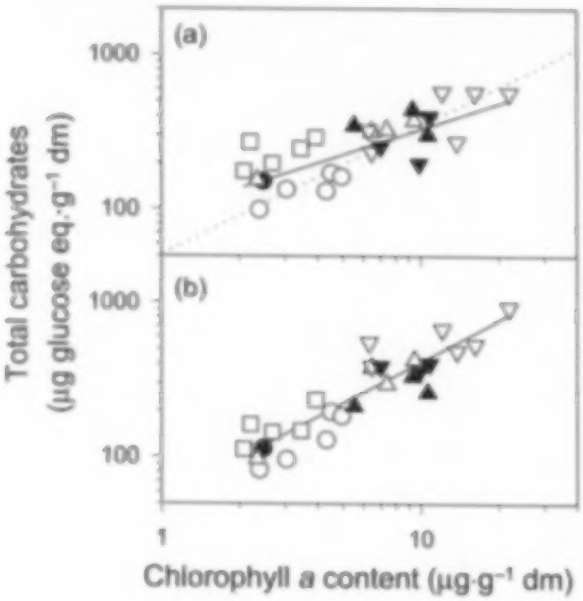
Table 2. Ranges of sediment carbohydrate (μg glucose equiv.·g^{-1} dm) and protein (μg BSA equiv.·g^{-1} dm) contents and of the percentages in large polymers (EPS) in each lake.

	Carbohydrates						Proteins					
	Colloidal			Capsular			Colloidal			Capsular		
Lake	Total	% as EPS	*n*	Total	% as EPS	*n*	Total	% as EPS	*n*	Total	% as EPS	*n*
Solitaire	176–292	6–11	5	112–234	9–19	5	246–527	5–8	5	625–1515	3–5	5
Harp	304–848	9–19	4	213–1016	10–20	4	585–2589	6–12	4	1157–8535	3–4	4
Annie Bay	99–173	8–15	6	83–196	9–26	6	235–806	4–9	6	559–1333	2–5	6
Halls	154–378	6–16	4	97–425	15–24	4	647–1304	5–7	4	954–3720	3–4	4
Plastic	236–577	9–20	6	388–927	13–23	6	1024–3228	4–6	6	4861–8594	3	6
Costello	136–152	11	2	113	11	1	242–392	5–8	2	960–1379	2	2
Paint	199–401	3–10	5	356–404	10–12	5	581–1874	2–4	2	3623–5096	2–3	2
Median		10			15			6			3	

Note: The total carbohydrate and protein measurements include monomers and polymers. dm, dry mass; equiv., equivalents; *n*, the number of sites at which total contents were measured.

Fig. 7. Relationship between chlorophyll *a* content and total carbohydrate content (i.e., monomers and polymers; TOTC$_i$) in the (*a*) colloidal and (*b*) capsular fractions in the shallow littoral zone of seven Canadian Shield lakes. The points are means for each site (among-core variability is not shown for clarity). Solid lines are the best fit model: $\log_{10}(\text{TOTC}_{\text{coll}}) = 1.97 + 0.55\log_{10}(\text{CHLA})$, $P < 0.0001$, $r^2 = 0.56$, $n = 28$; $\log_{10}(\text{TOTC}_{\text{caps}}) = 1.72 + 0.89\log_{10}(\text{CHLA})$, $P < 0.0001$, $r^2 = 0.82$, $n = 27$. Annie Bay (○), Costello (●), Halls (△), Harp (▲), Plastic (▽), Paint (▼), Solitaire (□). Broken line represents the model of Underwood and Smith (1998) for marine intertidal mudflats. dm, dry mass; eq., equivalents.



nificant effect of sediment mean particle size on the content of capsular carbohydrates ($P > 0.2$). We found no significant differences in these relationships between clear and dark lakes (ANCOVA using multiple regression, $P > 0.2$ on main effect of I and its interaction terms, see Materials and methods; compare open and solid symbols in Fig. 7).

Exopolymeric substances in littoral sediments

A small proportion of the carbohydrates and proteins in sediments were in large polymeric (EPS) form (Table 2). The EPS represented, on average (median), 10%–15% of the carbohydrates and 3%–6% of the proteins.

The colloidal EPS content of littoral sediments varied with light availability and, possibly, with sediment particle size (Fig. 8). Similar models describe the relationships for carbohydrates ($\log_{10}(\text{collEPS}_{\text{carbo}}) = 2.6 - 0.7\text{PHI}_{<2\text{mm}} - 1.6I + 0.7[\text{PHI}_{<2\text{mm}} \cdot I]$, $P = 0.0002$, $r^2 = 0.45$, $n = 32$; Fig. 8*a*) and for proteins ($\log_{10}(\text{collEPS}_{\text{protein}}) = 3.1 - 1.0\text{PHI}_{<2\text{mm}} - 1.9I + 0.9[\text{PHI}_{<2\text{mm}} \cdot I]$, $P = 0.004$, $r^2 = 0.32$, $n = 30$; Fig. 8*b*). These models suggest that more colloidal EPS is measured in the darker lakes (<20% I_0) when comparing sandy sediments ($\text{PHI}_{<2\text{mm}} = 1.7–2.0$), but less colloidal EPS is measured in these darker lakes when comparing muds ($\text{PHI}_{<2\text{mm}} = 2.3$). The interaction terms in

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Fig. 8. Relationships between sediment mean particle size ($PHI_{<2mm}$) and colloidal exopolymeric substances (EPS) content for (*a*) carbohydrates (EPS_{carbo}) and (*b*) proteins ($EPS_{protein}$) in lakes with low and high light penetration (<20% surface irradiance (I_0), $I = 0$; ≥40% I_0, $I = 1$). The points are means for each site (among-core variability is not shown for clarity). The best models describing these relationships are (*a*) $\log_{10}(EPS_{carbo}) = 2.6 - 0.7PHI_{<2mm} - 1.6I + 0.7[PHI_{<2mm} \cdot I]$ ($P = 0.0002$, $r^2 = 0.45$, $n = 32$); (*b*) $\log_{10}(EPS_{protein}) = 3.1 - 1.0PHI_{<2mm} - 1.9I + 0.9[PHI_{<2mm} \cdot I]$ ($P = 0.004$, $r^2 = 0.32$, $n = 30$). Solid symbols and dotted line for lakes with low light (models above with $I = 0$), open symbols and solid line for lakes with high light (models with $I = 1$). Annie Bay (○), Costello (●), Halls (△), Harp (▲), Plastic (▽), Paint (▼), Solitaire (□). fm, fresh mass; eq., equivalents.

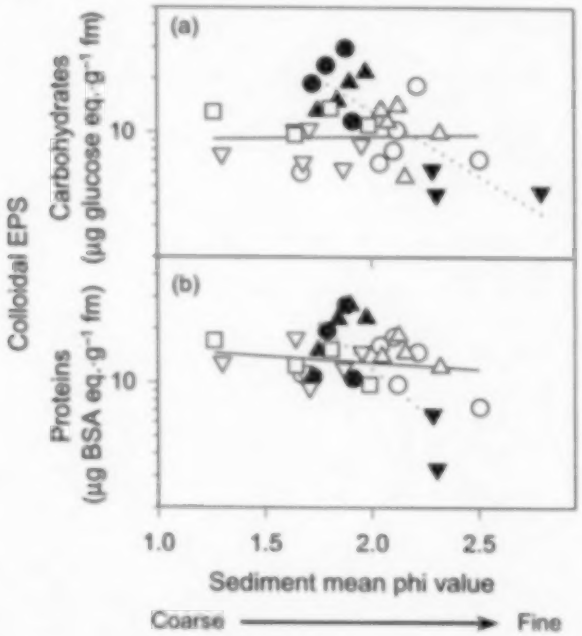
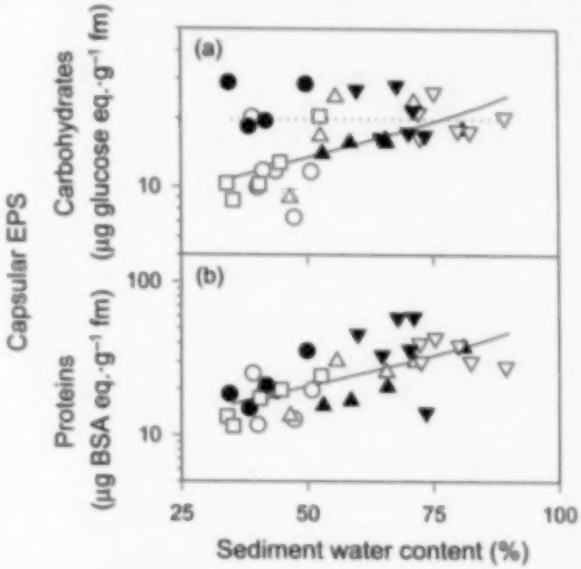


Fig. 9. Relationships between sediment water content (WC; axis with untransformed values) and capsular exopolymeric substances (EPS) content for (*a*) carbohydrates (EPS_{carbo}) and (*b*) proteins ($EPS_{protein}$) in lakes with low and high light penetration (<20% surface irradiance (I_0), $I = 0$; ≥40% I_0, $I = 1$). The points are means for each site. The best models describing these relationships are (*a*) $\log_{10}(EPS_{carbo}) = 1.3 - 0.6I + 0.01[I\text{-arcsine}(\sqrt{WC})]$ ($P < 0.0001$, $r^2 = 0.42$, $n = 3$; dotted line, lakes with low light; solid line, lakes with high light) and (*b*) $\log_{10}(EPS_{protein}) = 0.7 + 0.013\text{arcsine}(\sqrt{WC})$ ($P < 0.0001$, $r^2 = 0.41$, $n = 35$; solid symbols and dotted line for lakes with low light (model with $I = 0$); open symbols and solid line for lakes with high light (model with $I = 1$). Annie Bay (○), Costello (●), Halls (△), Harp (▲), Plastic (▽), Paint (▼), Solitaire (□). eq., equivalents.



these models are highly significant ($P \leq 0.002$) but should be interpreted with caution as they represent sites in only one lake (Paint Lake; Fig. 8). When these sites are excluded from the analysis, the effect of sediment particle size disappears, but the effect of light availability is still highly significant. Dark lakes have almost twice as much colloidal EPS carbohydrates (ANCOVA, $P < 0.0001$) and 40% more colloidal EPS proteins ($P = 0.01$) than clear lakes. The colloidal EPS content of littoral sediments was not related to sediment water content ($P > 0.3$).

The more tightly bound capsular EPS varied with sediment water content and light availability (Fig. 9) but not with mean particle size. The carbohydrate content was best described by the following model: $\log_{10}(capsEPS_{carbo}) = 1.30 - 0.6I + 0.010[WC \cdot I]$ ($P < 0.0001$, $r^2 = 0.42$, $n = 36$; Fig. 9*a*). This suggests that capsular EPS carbohydrate content did not vary with sediment water content in dark lakes (Fig. 9*a*) and was lower in the clearer lakes, but only on sediments with low water content (Fig. 9*a*). The capsular EPS carbohydrate content was not related to sediment particle size ($P > 0.1$) and was only weakly (and positively) related to sediment total phosphorus content ($P = 0.004$, $r^2 = 0.22$).

In contrast, the capsular EPS protein content varied with sediment water content but was not affected by light availability: $\log_{10}(capsEPS_{protein}) = 0.7 + 0.013WC$ ($P < 0.0001$, $r^2 = 0.41$, $n = 35$; Fig. 9*b*). Across all sites, there were clearly more capsular EPS proteins in sediments with higher water content. The capsular EPS protein content was not related to sediment particle size ($P > 0.1$) and was only weakly (and positively) related to sediment total phosphorus content ($P = 0.001$, $r^2 = 0.28$).

Sediment protein–carbohydrate ratios increased with increasing sediment water content in the capsular EPS fraction ($P < 0.0001$, $r^2 = 0.34$, $n = 35$; Fig. 10) but not in the colloidal fraction ($P > 0.1$; not shown).

Discussion

Extracellular carbohydrates and proteins in littoral sediments

The shallow littoral sediments of seven Canadian Shield lakes contained large amounts of carbohydrates and proteins.

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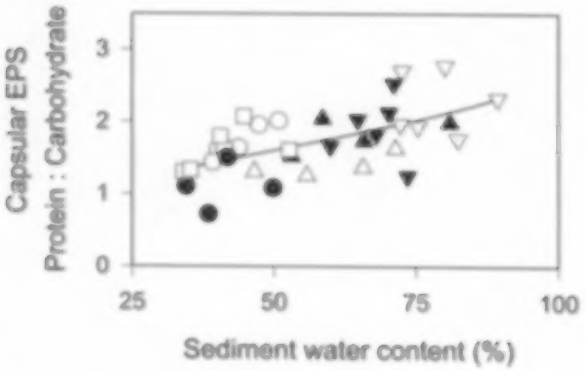
Fig. 10. Relationship between sediment water content (WC; axis with untransformed values) and capsular exopolymeric substances (EPS) protein to carbohydrate ratio ($PROT:CARB_{caps}$) in lakes with low and high light penetration (<20% surface irradiance (I_0), $I = 0$; ≥40% I_0, $I = 1$). The points are means for each site. Solid line is the best fit model: $PROT:CARB_{caps} = 0.3 + 0.028\text{arcsine}(\text{sqrt}(WC))$ ($P = 0.0001$, $r^2 = 0.34$, $n = 35$; solid symbols and dotted line for lakes with low light; open symbols and solid line for lakes with high light). Annie Bay (○), Costello (●), Halfs (△), Harp (▲), Plastic (▽), Paint (▼), Solitaire (□).



The colloidal fraction is perhaps the most comparable (at least functionally) between freshwater and marine studies because it is extracted simply with water. We measured colloidal carbohydrate contents between 100–200 and 850 µg glucose equiv.·g^{-1} dm in our samples. These measurements are similar to the values reported by Hirst et al. (2003) for a polymictic Canadian Shield lake basin. They are also within the range of values reported from marine intertidal flats (10–5400 µg glucose equiv.·g^{-1} dm; see review in Hirst et al. 2003), where the importance of extracellular production and EPS is well established (Underwood and Paterson 2003). Extracellular proteins are rarely measured in marine intertidal flats because they represent a small fraction of the extracellular releases from algae (Hoagland et al. 1993; Staats et al. 1999), which have been the focus of study. Microbial communities, however, have been shown to release large quantities of proteins (up to 60% of EPS), especially in nutrient-rich environments (Flemming and Wingender 2001). In this study, we measured large amounts of extracellular proteins in lake sediments.

Total carbohydrate contents varied an order of magnitude between the lakes we sampled. Within lakes, the colloidal carbohydrates varied only two- to three-fold between sampling sites. Capsular carbohydrates were similar at all sampling sites in some lakes (Paint Lake) and ranged as much as fivefold in other lakes (Harp Lake). The capsular fraction is generally considered to contain more recalcitrant substances that may accumulate in the sediments (van Duyl et al. 1999, 2000; de Brouwer et al. 2003). Differences in carbohydrate contents between sites and between lakes reflect differences in productivity, in lability, and in environmental conditions affecting the degradation of these compounds.

Between 3% and 25% of the carbohydrates in sediments were in large polymeric (EPS) form. This is a smaller proportion than what is measured in marine intertidal flats, where EPS represent 25%–45% of the colloidal carbohydrates (Underwood and Smith 1998; van Duyl et al. 2000) and 20%–40% of capsular carbohydrates (Underwood et al. 1995; Goto et al. 1999). Many laboratory studies have shown that the proportion of EPS produced by algae varies widely among species and is affected by the physiological status of the algae and by environmental conditions (e.g., Goto et al. 1999; Smith and Underwood 2000; Underwood et al. 2004). It is unclear at this point whether these apparent differences in the composition of carbohydrates between lake sediments and intertidal flats are due to differences in methodology (e.g., we used 60% ethanol to precipitate EPS compared with 70% in many marine studies), differences in the organisms present, and (or) differences in environmental conditions.

Algal biomass and sediment carbohydrate content

The colloidal and capsular carbohydrates were both strongly related to chlorophyll *a* content in the sediments, an indicator of algal biomass. These results are consistent with the findings of Underwood and Smith (1998), who demonstrated a close relationship ($r^2 = 0.64$) between the colloidal carbohydrate content of sediments and algal biomass (chlorophyll *a*) in intertidal mudflats (shown as dotted line in Fig. 7*a*). Their model was developed using an extensive data set from the Blackwater and Severn estuaries ($n = 277$), validated with data from three other UK estuaries, and was supported by several other studies (e.g., van Duyl et al. 1999; Staats et al. 2001; de Brouwer et al. 2003). Interestingly, based on the model of Underwood and Smith (1998), the colloidal carbohydrate contents in lake sediments are very similar to those measured in marine intertidal mudflats over the same range of chlorophyll *a* content (compare symbols with dotted line in Fig. 7*a*).

The relationship between colloidal carbohydrates and chlorophyll *a* in intertidal mudflats is attributed either to the migration of diatoms through the sediments (Underwood and Paterson 2003) or to an overflow of excess photosynthates when the algae are exposed to high light and (or) poor nutrient conditions (e.g., Staats et al. 2000*a*; Underwood 2002). In lakes, a few studies have reported migration of epipelic algae (diatoms, flagellates, cyanobacteria) through sediments (Round and Eaton 1966; Harper 1969; Wasmund and Kowalczewski 1982). These few observations need to be tested more generally and with more precise techniques to determine whether algal migration is common in lake sediments and whether it is regular (e.g., diurnal). The second explanation invoked in intertidal mudflats (overflow of excess photosynthates) cannot fully explain the pattern we are finding. On a bright summer day (2200 µmol photons·m^{-2}·s^{-1}), the sediments in the clearest lakes we sampled receive 900–1200 µmol photons·m^{-2}·s^{-1} compared with 300–400 µmol photons·m^{-2}·s^{-1} in the darkest lakes. Overflow of excess photosynthates have been shown at much lower light levels under low nutrient conditions (e.g., Otero and Vincenzini 2004), but we found no effect of light availability on the total carbohydrate content measured in sediments. A third possible explanation is the passive release by algae of a portion of their primary production (Bjørnsen 1988; Maranon et al.

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2004). Our data do not allow us to distinguish between these hypotheses.

The model proposed by Underwood and Smith (1998) to predict colloidal carbohydrates from chlorophyll *a* measurements has been criticized because it does not apply generally to marine intertidal flats. Significant relationships between colloidal carbohydrates and chlorophyll *a* are only found in mudflats dominated by motile diatoms (Underwood and Smith 1998). These relationships are not found on sandflats (Underwood and Smith 1998; Blanchard et al. 2000) and do not apply to areas of mudflats where the colloidal compounds are washed away (Blanchard et al. 2000). Sandier sites are usually dominated by cyanobacteria and small, attached diatoms (Watermann et al. 1999; Sundback et al. 2000; Stal 2003), which release more tightly bound carbohydrates. In contrast, the relationships we report for lake sediments apply to a wide range of sediment types and to communities with different taxonomic composition.

We also found a close relationship between the more tightly bound (capsular) carbohydrates and chlorophyll *a*. Similar relationships have been reported in some studies (e.g., Battin and Sengschmitt 1999; van Duyl et al. 1999), but not others (e.g., Staats et al. 2001; de Brouwer et al. 2003). Several studies have suggested that the capsular fraction contains refractory compounds that are less directly related to algal productivity than colloidal compounds (e.g., van Duyl et al. 2000; de Brouwer and Stal 2001). However, data on the capsular fraction are difficult to compare, especially between freshwater and marine studies, because the tightly bound fraction being measured depends on the extraction procedure being used, and these procedures differ widely among research groups. In the shallow littoral zone of lakes, the colloidal and capsular carbohydrates are both related to chlorophyll *a*, and the capsular fraction is even more closely related to chlorophyll *a* than the colloidal fraction ($r^2 = 0.87$ and 0.56, respectively). The relatively weak physical forces in small lakes (compared with intertidal flats, rivers, and streams) may allow the accumulation of recalcitrant capsular compounds in littoral sediments.

Effects of light, nutrients, and sediment type on EPS

Light availability and sediment type affect the content and composition of EPS in littoral sediments in complex ways. The loosely bound colloidal EPS in littoral sediments differed between clear and dark lakes and were possibly related to sediment type in darker lakes. The results were qualitatively similar for carbohydrates and proteins.

We found more colloidal EPS on sandy substrates (mean phi value = 1.7–2.0 or average particle size = 250–300 µm) in dark lakes than in clear lakes, but on finer sediments, dark lakes had less colloidal EPS than clear lakes (if the data from Paint Lake are representative). Light limitation has been shown to increase the release of extracellular compounds in phytoplankton (e.g., Maranon et al. 2004) and in benthic algal cultures (e.g., Smith and Underwood 2000). Perkins et al. (2001) performed a shading experiment on a marine intertidal mudflat and reported a 2.5 times increase in colloidal EPS carbohydrate production rate under 50% light reduction. Reduced light availability in dark lakes is expected to increase colloidal EPS production. However, the dark lakes also have high dissolved organic carbon (DOC) concentrations in the water column, which could affect indirectly the production and (or) the degradation of EPS in the sediments. The photodecay of DOC into dissolved inorganic carbon (DIC) could possibly enhance primary productivity in shallow littoral areas (Molot and Dillon 1997*b*), enhancing the production of EPS. On the other hand, sedimentary areas in dark lakes may be enriched in DOC (e.g., higher allochthonous inputs, flocculation of DOC, absorption of DOC by highly organic sediments; Stewart and Wetzel 1981; Weilenmann et al. 1989), which would enhance microbial processes and possibly the degradation of EPS. The differences we found in the colloidal EPS content of littoral sediments between clear and dark lakes could be due to differences in light availability and (or) indirect effects of higher DOC concentrations.

Sediment type affects the colloidal EPS content, but only in dark lakes. There was no effect of sediment particle size on colloidal EPS content in lakes with high light availability in the littoral zone (≥40% surface irradiance). In darker lakes, there was a sharp decrease of colloidal EPS in finer sediments, assuming that data from Paint Lake are representative. These results are likely to reflect complex interactions between the algal and microbial communities and could be interpreted in several ways. For example, microbial communities in finer sediments (or in Paint Lake sediments) may be more active and more efficient at breaking down polymeric substances. It would also not be surprising if the EPS produced by benthic communities in different types of substrates differed in its composition. Our results differ from the patterns observed in marine intertidal flats, where finer sediment particles contain more colloidal carbohydrates than coarser particles (e.g., de Brouwer et al. 2003). However, patterns in the distribution of colloidal compounds are difficult to interpret because these compounds are very labile and may not accumulate in the sediments (Taylor et al. 1999; de Brouwer and Stal 2001). These patterns in the distribution of colloidal EPS in littoral sediments are suggestive and merit further investigation.

In contrast to colloidal EPS content, the more tightly bound (capsular) EPS were not significantly related to light availability or sediment particle size but increased weakly with increasing total phosphorus content of the sediments ($r^2 = 0.22–0.28$, $P < 0.005$). The relationships with phosphorus are highly significant (statistically) and are qualitatively similar for carbohydrates and proteins. Many studies have shown that nutrient limitation in algae and bacteria increases the release of EPS (e.g., Decho 1990; Staats et al. 2000*a*; Underwood et al. 2004). However, chlorophyll *a* content (a measure of algal biomass) was also positively related to sediment total phosphorus content (regression with chlorophyll expressed in µg g^{-1} dm, $r^2 = 0.33$, $P < 0.001$), so this positive relationship is likely driven by other factors.

The capsular EPS content was positively related with sediment water content. Carbohydrates were significantly related to sediment water content in clear lakes, but not in dark lakes. Light-limited algae have been shown to release more carbohydrates (Perkins et al. 2001), but the increase in EPS is only observed in compact sediments (water content <50%) with low organic matter content. Positive relationships between capsular EPS carbohydrates and sediment water content have also been reported in intertidal mudflats (e.g., Yallop et al.

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2000, in which capsular EPS are called Coll-EDTA EPS). The proteins were related to sediment water content, regardless of light availability. This relationship is not surprising if bacteria are the main producers of EPS proteins. Microbial productivity is very tightly related to sediment organic matter content (Sander and Kalff 1993; Hirst et al. 2003), which is itself related nonlinearly to sediment water content. However, because algal biomass and sediment organic matter content both increase with increasing sediment water content, it is impossible to clearly dissociate the role of algae and bacteria in these relationships.

We measured protein–carbohydrate ratios as a rough indicator of the potential quality of EPS as food source for benthic invertebrates. The protein–carbohydrate ratios of colloidal and capsular EPS were much higher than in detritus, approaching ratios measured in live algae (Lamberti 1996). These proteins could be important to littoral foodwebs, as the growth of benthic invertebrates is limited, at least in part, by the availability of nitrogen (e.g., Vos et al. 2000). However, it is still unclear whether these proteins are available to invertebrates. Couch et al. (1996) found that intact EPS were assimilated more efficiently by black fly (Simuliidae) larvae than EPS from which proteins had been extracted. In contrast, Decho and Lopez (1993) reported low assimilation of EPS proteins by a marine polychaete. Both studies used microbial EPS produced under a limited range of conditions, and it is unclear how these results apply to EPS with different chemical composition and to other benthic feeders.

Exopolymeric carbohydrates and proteins have been shown to play a suite of important roles in ecosystems (Decho 1990, 2000; Underwood and Paterson 2003). We found large concentrations of these substances in the littoral sediments of several Canadian Shield lakes, confirming and extending the findings of Hirst et al. (2003). The functional role of these extracellular substances in the productivity and nutrient dynamics of shallow littoral zone of lakes remains to be tested.

Sediment water content: an indicator of biological processes

Sediment water content is a simple measure of sediment texture, which is closely related to important sediment characteristics, such as organic and nitrogen content. Relationships between sediment water content and organic content have been reported in other lakes (e.g., Håkanson and Jansson 1983; Cyr 1998), estuaries (e.g., McComb et al. 1998), and oceans (e.g., Carman et al. 1996; Christie et al. 2000). It is also well known that most of the nitrogen in sediments is associated with the organic matter (e.g., Håkanson 1984; Carman et al. 1996; McComb et al. 1998).

Somewhat surprisingly, sediment water content in shallow littoral sediments is not related to mean particle size. This contrasts with the strong relationships reported from deeper areas in lakes (e.g., Håkanson and Janson 1983; Rowan et al. 1992). At wind-protected sites, the shallow littoral sediments can have very high water content (Cyr 1998) but are composed of much coarser particles (mean phi value = 1.5–2.5) than the fine silts and clays that accumulate deeper in lakes (e.g., phi value = 6–7 in sediments with 70%–90% water content; Rowan et al. 1992). Sediment water content is determined not only by the size of sediment particles, but also by the degree of compaction of these sediments.

Sediment water content is a good predictor of organic content, algal biomass, capsular EPS content, and the capsular protein–carbohydrate ratio in littoral sediments. Sediment porewater content is very simple and quick to measure, and new developments in hydroacoustics are already being used to rapidly survey and map the sediment texture (hard substrate, sand, mud) at the bottom of lakes and oceans (e.g., SeaMap Program). These sediment-type maps are potentially useful tools for managing biological resources, but their usefulness depends on our capacity to predict ecological variables from sediment texture. Sediment porewater content offers promises of quick assessments of the notoriously heterogeneous littoral zone of lakes to determine, for example, which parts of the sedimentary littoral zone are most productive.

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Contrasting deterministic and probabilistic ranking of catch quotas and spatially and size-regulated fisheries management

P. Apostolaki, E.A. Babcock, and M.K. McAllister

Abstract: Large uncertainties over the dynamics of resource systems have increasingly led to the use of probabilistic modeling in the provision of model-based fishery management advice. However, deterministic analysis still remains the easiest and quickest approach to formulate model-based management advice. Here, we contrast deterministic and probabilistic modeling methods in evaluations of the potential consequences of alternative fishery management measures such as spatial and temporal closures and size-specific regulations. We thereby assess how model-based fishery management advice may vary between deterministic and probabilistic analyses of system dynamics. Using data for the sandbar shark (*Carcharhinus plumbeus*) population off the eastern coast of the USA, it is shown that under a variety of conditions, the use of management measures that provide protection to specific age groups of a population, such as size limits, might be less effective in achieving stock recovery of slow-growing, late-maturing, highly mobile species than catch quotas. It is also shown that management approaches that, according to deterministic calculations, appear to be the most effective are not so when uncertainty in the population dynamics is taken into account.

Résumé : Les incertitudes importantes dans la dynamique des systèmes de ressources naturelles ont favorisé l'utilisation de plus en plus répandue de la modélisation probabiliste pour générer des avis dans la gestion des pêches commerciales à l'aide de modèles. Cependant, l'analyse déterministe reste la méthode la plus facile et la plus rapide pour produire des avis concernant la gestion par modèles. Nous comparons ici des méthodes de modélisation déterministes et probabilistes pour l'évaluation des conséquences potentielles de mesures de rechange de gestion des pêches, telles que des fermetures spatiales ou temporelles de la pêche et des réglementations reliées à la taille des poissons. Cela permet d'évaluer les différences dans les avis produits par des analyses déterministes et probabilistes de la dynamique des systèmes. L'étude de données sur une population de requins gris (*Carcharhinus plumbeus*) au large de la côte est des É.-U. montre que, dans une variété de conditions, l'utilisation de mesures de gestion qui visent à protéger des groupes d'âge spécifiques de la population, telles que des limites de taille, peuvent être moins efficaces que les quotas de captures chez les espèces à croissance lente, à maturité tardive et à forte mobilité. Elle montre aussi que les approches de gestion qui, dans les calculs déterministes, semblent être les plus efficaces ne le sont plus lorsqu'on tient compte de l'incertitude dans la dynamique de la population.

[Traduit par la Rédaction]

Introduction

Many studies have stressed that incomplete knowledge of the system of interest is unavoidable in fisheries science; therefore, fishery managers need to allow for errors in stock assessment and utilize formal methods of risk evaluation to decide upon fishery management strategies (Peterman and Anderson 1999; Hilborn et al. 2001; Mangel et al. 2002). However, accounting for uncertainty requires the use of sophisticated techniques and computationally demanding calculations (Punt and Hilborn 1997), whereas deterministic analysis and simulation of the marine resource systems is much simpler and quicker. Consequently, deterministic simulation models have been and continue to be widely used to assess marine as well as terrestrial resources (Mollet and Cailliet 2002). Thus, it is natural to ask whether the results

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of deterministic analysis could be relied upon to identify effective strategies for fisheries management.

Although some fishery scientists have addressed this issue, they have generally done so by choosing a fishery control rule (e.g., catch quota, harvest effort quota) and calculating the changes in the value of the performance indicator that would be caused by the introduction of uncertainty in the analysis. For example, Tanner (2003) compared the deterministic predictions of his model (the effectiveness of closing certain areas of the fishing ground for a prawn fishery) with the predictions of the model when uncertainty in the parameter values had been taken into account. Tanner found that "stochastic variation in a single parameter had very little effect on the optimal harvest regime" (Tanner 2003) and that varying natural mortality caused the greatest effect. The same observations held when stochasticity was added to the values of more than one parameter of the model (e.g., natural, mortality, juvenile survival, fecundity). Thus, he concluded that for the specific case examined, the optimal harvest regime predicted using a deterministic model would not be altered by the addition of stochastic variation to the model. McDonald et al. (2002) compared the economically optimal harvest levels that would be adopted for southern bluefin tuna (*Thunnus maccoyii*) if deterministic feedback rules were used to define harvest levels with those found when stochastic rules were applied. He concluded that the stochastic feedback rules can lead to more caution at moderate or high population levels, but that the harvest levels that would be chosen on the basis of the deterministic model were less likely to drive the stock to extinction than those based on the stochastic analysis. Overall, the authors found that under certain conditions (e.g., low discount rates), the deterministic approach performs quite well. Similarly, Frederick and Peterman (1995) calculated allowable harvest levels using the best point estimates of abundance and stock–recruitment parameters and compared their results with model estimates about harvest levels when uncertainty was taken into account. They found that harvest decisions that were based on results of deterministic analysis could often approximate an optimal strategy. However, they pointed out that the difference between the optimal strategy based on deterministic analysis and that found when uncertainty was taken into account is likely to be considerable when the size of the stock is below a biological threshold. Our study will extend existing research by evaluating the changes in perceptions about the effectiveness of a suite of fishery management strategies caused by the introduction of uncertainty in the analysis.

There is considerable uncertainty about the biological processes and dynamics for most exploited shark populations (Bonfil 1994; Stevens et al. 2000*a*; Courtney et al. 2004). This lack of information is largely a consequence of the relatively low economic value of sharks (Walker 1998). However, shark catches have increased dramatically in the last few decades, and there is a growing concern regarding the impact of fishing on shark populations (FAO 1999; Stevens et al. 2000*a*; Worm et al. 2003). Owing to the limited biological and exploitation information for exploited sharks, predictions about population status and the potential consequences of alternative management measures have in several cases been based on results of deterministic analyses or are characterized by high uncertainty when stochastic analysis is used (Smith et al. 1998; Simpfendorfer et al. 2000; Cortés et al. 2002; but see Punt and Walker 1998).

This study applies probabilistic and deterministic analyses to address three questions: (*i*) What are useful approaches to the provision of model-based advice for the management of fisheries where few data exist on the system of interest? (*ii*) How might perceptions about the most effective fishery management approach change when uncertainty is incorporated in the assessment process? (*iii*) Which management measures can be expected to perform well under a range of plausible scenarios for population biology and dynamics when there are little data and high uncertainty?

The analyses and simulations that we have constructed to address these questions are structured around data for the commercially important sandbar shark (*Carcharhinus plumbeus*) population off the eastern coast of the USA. An age- and sex-structured model is employed to simulate the dynamics of the sandbar shark population. Bayesian statistical methods are used to incorporate uncertainty in the analysis. We use this statistical modeling framework to evaluate the potential effects of fishery management measures, such as catch quotas, legal minimum length, and spatial closures, under deterministic and probabilistic decision analysis frameworks. The results of the deterministic and probabilistic analyses are used to examine the extent to which the provision of management advice is sensitive to uncertainty in stock assessment predictions and to evaluate and compare the effectiveness of alternative fishery management schemes.

Materials and methods

Statistical modeling framework

An age- and sex-structured, gear-disaggregated, spatially explicit model is used for the simulation of the population dynamics. The use of an age- and sex-structured model allows for incorporation of information about changes in important biological processes with age or sex. For example, age- and sex-structured models can account for fishing effort that is not uniformly distributed across the age classes and allow for the incorporation of age-specific catch rate information. Similarly, when there are sex-specific differences in growth rates or exposure to fishing, age- and sex-structured models can appropriately reflect those differences.

The model is designed to calculate the number of fish in each age class at each time step while taking into account natural and fishing mortality as well as the reproductive features of the population. The model also simulates spatial and temporal heterogeneity in the distribution of fish. A fine temporal scale (3 months) is used for the calculations to increase the realism in the simulation of fish movement and population dynamics. The model assumes that the managed area consists of two subareas and uses movement vectors to describe the movement of fish between the subareas. Probability density functions (pdfs) are used to express a priori uncertainty in the values of the estimated model parameters, such as the virgin population biomass of the population and the parameters of the stock–recruitment function. For the assessment of the current status of the stock, it is assumed that catch data are known without error, while the observed catch-per-unit-effort (CPUE) values are assumed to be log-normally

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distributed about the model-predicted values. A detailed description of the assumptions and equations used for the development of the population dynamics model and Bayesian modeling framework follows.

Population dynamics model

The population dynamics model calculates the number of fish ($N^{e}_{g,y,t,a}$) in age class a at the end of each 3-month time step t as follows:

$$(1)\quad N^{e}_{g,y,t,a} = \begin{cases} N_{g,y,1,0} & a = 0, t = t_{pup} \\ (N^{b}_{g,y,t,a}\, S_a^{1/8} - C_{g,y,t,a})\, S_a^{1/8} & \text{otherwise} \end{cases}$$

where g is gender and y is year. $N^{b}_{g,y,t,a}$ is the number of fish of sex g in age class a at the beginning of time step t. S_a is the annual survival rate for age a fish. $C_{g,y,t,a}$ is the number of fish of sex g in age class a that were caught at time step t in year y. $N_{g,y,t_{pup},0}$ is the number of pups of gender g born in time step t in year y and is equal to $f_g N_{0,y}$, where $N_{0,y}$ is the total number of pups born in time step t_{pup} (the time step when pupping takes place) in year y, and f_g is the fraction of pups of sex g. It is assumed that pupping takes place at the end of the time step that includes the pupping season, which is assumed to be summer for sandbar shark, and pups could be vulnerable to fishing beginning in the next time step.

Since the time step used is equal to 3 months, the number of fish caught at time step t in year y with gear j ($C_{y,t,j}$) is equal to one-fourth of the corresponding annual catches unless nonuniform temporal distribution of fishing is simulated. Following Punt and Walker (1998), catches are assumed to be taken in a pulse in the middle of each time step after the population has experienced natural mortality for half of one time step:

$$(2)\quad C_{g,y,t,a,j} = \left(N^{b}_{g,y,t,a}\, S_a^{1/8} - \sum_{j'=1}^{j-1} C_{g,y,t,a,j'} \right) v_{g,a,j}\, u_{y,t,j}$$

where $v_{g,a,j}$ denotes vulnerability of fish of age a and sex g to fishing gear j (gear selectivity), and $u_{y,t,j}$ is the exploitation rate for fishing gear j at time step t. If the catch (number of fish) per fishing period and gear are known, the exploitation rate is

$$(3)\quad u_{y,t,j} = \frac{C_{y,t,j}}{\sum_{g}\sum_{a} v_{g,a,j} \left(N^{b}_{g,y,t,a}\, S_a^{1/8} - \sum_{j'=1}^{j-1} C_{g,y,t,a,j'} \right)}$$

Fish weight at age a is assumed to be a function of fish length $L_{y,a}$. Fish length at age is calculated using the von Bertalanffy growth equation.

The model uses a stock–recruitment function to calculate the survival of pups from natural causes of death during the first year of their life. Density-dependent population regulation at early stages can be included through the stock–recruitment function; it is assumed that density-dependent processes are not characteristic of older age classes. The Beverton–Holt stock–recruitment function was chosen to calculate sandbar shark pup survival (Beverton and Holt 1957). This function has been proposed as an appropriate stock–recruitment relationship for shark species (Walker 1998) and has already been used in modeling studies of other shark species (Simpfendorfer et al. 2000; Campana et al. 2001; Brooks et al. 2002). It was considered an appropriate choice for sandbar shark given that sandbar shark produces few offspring, and for a considerable part of the year, pups do not share the same areas with mature sandbar sharks, thus reducing the chance of cannibalism. Using the Beverton–Holt relationship, the survival of fish during the first year of their life ($S_{0,y}$) is expressed as a ratio:

$$(4)\quad S_{0,y} = \frac{R_y}{N_{0,y}} = \frac{1}{\alpha + \beta N_{0,y}}$$

where α and β are constants, $N_{0,y}$ is the number of pups born in year y, and R_y is the number of fish that were born in year y and are alive after a year. Taking the first derivative of R with regard to population size N, one can show that the survival of pups from natural causes of death at low population sizes is equal to $1/\alpha$.

The model allows for multi-annual reproductive cycles with one-half of the sexually mature females giving birth in each year. The number of pups born in year y is

$$(5)\quad N_{0,y} = \frac{1}{2}\sum_{a} N_{g=\text{fem},y,t=t_{pup},a}\, \phi_{g=\text{fem},a=1}\, \bar{\Phi}_a$$

where $\phi_{g,a}$ is the proportion of fish of age a and sex g that are mature, and $\bar{\Phi}_a$ is the average number of pups per age a pregnant female. A logistic curve is used to describe the proportion of fish at age a that are mature:

$$(6)\quad \phi_{g,a} = \frac{1}{1 + e^{(-k_g(a - a_{50_g}))}}$$

where a_{50_g} is the age at 50% maturity, and k_g is a constant that can be calculated if the ages at 50% and 95% maturity are known (but see Walker 2005 for a discussion on alternative ways to model shark reproduction, especially for species with multi-annual reproduction cycles).

Virgin conditions

If the survival at age and the total number ($N_{y_{vir},a}$) or biomass ($B_{y_{vir},a}$) of fish before any exploitation takes place is known, the number of fish of age a at the beginning of year y_{vir} can be calculated as

$$(7)\quad N^{b}_{g,y_{vir},t=1,a} = \begin{cases} f_g\, N_{0,y_{vir},t=t_{pup}}\, S_a^{1/2} & a = 0 \\ f_g\, R_{y_{vir},t=t_{pup}} \prod_{a'=1}^{a-1} S_{a'}\, S_a^{1/2} & 1 \le a \le a_{\max} - 1 \\ f_g\, R_{y_{vir},t=t_{pup}} \dfrac{\prod_{a'=1}^{a_{\max}-1} S_{a'}}{1 - S_{a_{\max}}}\, S_{a_{\max}}^{1/2} & a = a_{\max} \end{cases}$$

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where $R_{\bar{y}_{\text{virg}},r=1}$ denotes number of recruits under virgin conditions and is calculated from the total number or biomass of fish of age-1 or older under virgin conditions:

$$(8)\quad R_{\bar{y}_{\text{virg}},r=1} = \frac{B_{\bar{y}_{\text{virg}},r=1}}{\sum_g f_g \left(w_{g,a=1} + \sum_{a=2}^{a_{\max}-1} w_{g,a} \prod_{a'=1}^{a-1} S_{a'} + w_{g,a_{\max}} \frac{\prod_{a'=1}^{a_{\max}-1} S_{a'}}{1 - S_{a_{\max}}} \right)}$$

The number of pups born under virgin conditions is calculated using eq. 5 once the number of fish in each age class has been projected.

Movement

Heterogeneity in the spatial distribution of fish is simulated by assuming that the managed area consists of two subareas and by using movement coefficients to describe the movement between subareas. The expressions used to calculate the number of fish that occupy each area at each time step depend on the character of the two subareas. For example, if one of the two subareas is a nursery ground (area n), then we would need equations to describe the movement of immature fish into and out of this area. If $T_{g,t,a}(r,n)$ and $T_{g,t,a}(n,r)$ give the fraction of the population of sex g in age class a at time t that move from the area outside the nursery ground (area r) to the nursery ground (area n) and from the nursery ground to area r, respectively, then the number of fish in each of the two subareas is

$$(9)\quad N^{b}_{g,y,t,a,r} = \begin{cases} 0 & a = 0,\ t = t_{\text{pup}} + 1 \\ N^{a}_{g,t-1,a-1,r}\, T_{g,t,a}(n,r) + N^{a}_{g,y,t-1,a-1,r}\,(1 - T_{g,t,a}(r,n)) & a > 0 \end{cases}$$

$$(10)\quad N^{b}_{g,y,t,a,n} = \begin{cases} f_g\, N_{0,y} & a = 0,\ t = t_{\text{pup}} + 1 \\ N^{a}_{g,t-1,a-1,n}\,(1 - T_{g,t,a}(n,r)) + N^{a}_{g,y,t-1,a-1,r}\, T_{g,t,a}(r,n) & a > 0 \end{cases}$$

Movement of fish between the two subareas is assumed to be instantaneous at the end of each time step. $N^{a}_{g,y,t,a}$ is calculated by applying eq. 1 to each of the two subareas.

Likelihood function

Following McAllister and Kirkwood (1998), the log-likelihood function for all sets of observed values of the observed index data and for one potential set of values of the parameters of the model θ_m is

$$(11)\quad \ln L(I|\theta_m) = \sum_j \sum_k \sum_y -\frac{1}{2\sigma^2} \left(\ln \frac{I_{j,k,y}}{q_{j,k} B_{j,y,k}} \right)^2 - \ln\left(\sqrt{2\pi}\, I_{j,k,y}\, \sigma\right)$$

The observed values $I_{j,k,y}$ of each relative abundance series k from fishery j are assumed to be log-normally distributed about the corresponding model-predicted value. The relative abundance series used in our analysis are all CPUE series. A constant of proportionality ($q_{j,k}$) is used to express the model-predicted values ($B_{j,y,k}$) in the units used for the corresponding relative abundance series. σ is the log-normal standard deviation for residual errors between the observed and predicted values of relative fish abundance. It has been assumed that the variance is the same for all the points of the CPUE series; thus, σ^2 is constant and is treated as an estimated parameter of the model.

Bayesian integration

We use Bayesian statistics to incorporate and describe uncertainty and as a mechanism for incorporating prior information in the analysis. The prior pdfs used for the uncertain parameters of our model are discussed in the next section.

The integral of the joint posterior pdf with respect to each of the estimated parameters of the model cannot be found analytically; therefore, we used the sampling – importance resampling (SIR) algorithm to obtain numerical solutions. The SIR algorithm uses Monte Carlo techniques to approximate the posterior distribution in high-dimensional problems (Rubin 1987) and has been applied in Bayesian fisheries stock assessments (Raftery et al. 1995; McAllister and Ianelli 1997). The prior pdf was used as the importance function, and more than 50 000 draws were taken from the importance function in the first step of the SIR algorithm. In each draw from the importance function, the importance weight computed was the likelihood function of the data given the parameter values drawn from the importance function. Following McAllister and Ianelli (1997), the maximum percent weight of the largest importance weight over the sum of the weights was required to be less than 0.5%, so that any one draw had less than 0.5% influence on the estimate of the posterior pdf.

Study population

The sandbar shark belongs to the family Carcharhinidae and is a highly migratory, coastal–pelagic shark species found in warm-temperate and tropical oceans (Garrick 1982; Compagno 1984). Sandbar sharks are slow growing, *K*-

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Table 1. Values of the input parameters of the model.

Parameter	Value	
	Female	Male
Age at 50% maturity (a_{50}; years)[a]	13	12
Age at 95% maturity (a_{95}; years)[a]	15	14
a_{max} (years)[b,c]	35	
Survival from natural causes of death[b,d]	Varies from 0.75 year^{-1} for age 1 to 0.9 year^{-1} for fish older than 9 years	
Constants of the von Bertalanffy growth equation[e]		
K	0.059	0.059
L_∞ (cm)	197	184
t_0 (years)	–4.8	–5.4
Constants of the weight at length equation[c]		
b	3.0124	
a (kg·cm^{-1})[b]	1.0885×10^{-5}	
Fecundity[b,f,g]	9 pups every 2 years (sex ratio 1:1)	
Gestation period[f,h]	1 year	

[a]Sminkey and Musick (1995).
[b]Sminkey and Musick (1996).
[c]Casey and Natanson (1992).
[d]Cortés (2002).
[e]Kohler et al. (1996).
[f]Cliff et al. (1988).
[g]Castro (1993).
[h]Musick et al. (1993).

selected species that mature late. Female sandbar sharks are viviparous and give birth to few offspring (usually 5–12) every second year after a gestation period of 8–12 months (Cliff et al. 1988). The pupping season can start as early as May and continues during summer. Pregnant females utilize areas near the coast to give birth and then move offshore. Pups remain in these nearshore areas for the summer and part of autumn before they migrate to their wintering habitats (Merson and Pratt 2001). Young sandbar sharks continue to utilize the nursery areas and areas around them as summer habitats for the first 4–10 years of their life (Grubbs et al. 2006).

In the western North Atlantic, sandbar sharks exhibit an annual migration cycle along the eastern coast of the USA. They migrate south to overwintering areas below North Carolina and off the coast of Florida in the autumn and return to the north in May and early June (Compagno 1984). Sandbar sharks are an important component of the historical and current recreational and commercial fishery along the Atlantic coast of the USA and in the Gulf of Mexico (Casey et al. 1985; NMFS 2001). The commercial fishery targets sandbar sharks because of their high fin to carcass ratio. In addition, sandbar sharks are the second most often caught shark species in the recreational fishery (Sminkey and Musick 1995).

The management of the fisheries targeting or affecting the Atlantic shark resources within the US Economic Exclusive Zone is governed by the Fishery Management Plan for Atlantic Tunas, Swordfish, and Sharks (NMFS 1999). Depending on the area where fishing is taking place, the management of fisheries that target sandbar sharks could also be subject to management by state authorities, the Atlantic States Marine Fisheries Commission, and the relevant regional Fisheries Management Councils (i.e., Mid-Atlantic, South-Atlantic, and Gulf of Mexico Regional Fisheries Management councils). This jurisdictional overlap could complicate the implementation of fisheries management measures. Catch quotas are currently in place for the management of commercial as well as recreational fisheries. However, stock assessments conducted in recent years have shown that overexploitation of the sandbar shark population off the US east coast could be occurring (Cortés et al. 2002).

Implementation

Data and Bayesian priors

The biological information for the sandbar shark population that was used for the calculations is shown (Table 1). Commercial and recreational catch data from 1981 onwards were available (shown in Fig. 1). However, estimates of commercial catches between 1981 and 1985 were derived using indirect methods of calculation (e.g., fishermen's private records) rather than the official catch records. For this reason, two different catch data sets were used in the analysis: one that included catches between 1986 and 2001 and one that included catches between 1981 and 2001. Since there was little fishing for sandbar sharks before the mid-1970s, the starting year for the calculations was 1974, and the size of the population was assumed to be equal to its virgin size before that. Although exploitation of the sandbar shark population was taking place between 1974 and 1981, catch data are not available for that period. To account for the changes that might have occurred in the population size during that period (1974–1980 or 1974–1985), two simple scenarios for the catches between 1974 and the first year for which catch data exist were considered. It was assumed that the catches during that period were either constant or increased linearly.

Seventeen CPUE series (11 fishery-independent and 6 fishery-dependent) were available for use as indices of abundance for sandbar shark. A brief description of the main characteristics of each series is given below (see Cortés et al. 2002

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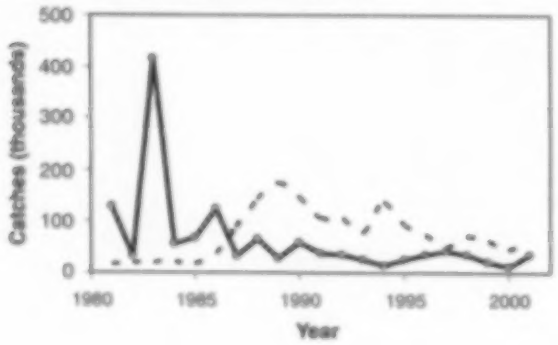
Fig. 1. Commercial and recreational catches (number of fish) per year for sandbar shark (*Carcharhinus plumbeus*) off the eastern coast of the USA (Cortés et al. 2002). Broken line, commercial catches (including Mexican and estimates of unreported catches); solid lines with circles, recreational catches.



for a detailed description of the series and information about where the results of the surveys can be found). Six of the fishery-independent CPUE data series came from the Virginia longline surveys (Cortés et al. 2002). Two of the Virginia longline survey CPUE series represented all ages (one in biomass and one in number of fish) for the period from 1974 to 2000. The remaining four series were age-specific and only provided estimates for some of the years in the period from 1980 to 2000. Three additional fishery-independent CPUE series were available from the longline surveys conducted by the National Marine Fishery Service for only some of the years between 1990 and 2001. Two fishery-independent CPUE series were developed based on data from the South Caroline longline surveys: one for the period from 1995 to 2001 and one for the period from 1983 to 1994. Also, two fishery-dependent CPUE series from the commercial US fishery were available: one for the period 1994–2001 and one for the period 1996–2001. Three additional fishery-dependent CPUE series were developed based on data from the recreational US fishery for the periods 1981–1993, 1994–2000, and 1986–2001. The information in the age-specific CPUE series from the Virginia longline survey is also included in the Virginia longline CPUE series, which represents all age classes. For this reason, either the age-specific CPUEs from the Virginia longline survey or one of the age-aggregated Virginia longline CPUE series were used together with the remaining CPUE series. For each of the simulation model runs, a different set of assumptions was made about the combination of CPUE data series (Virginia longline age-aggregated or not), the historical exploitation (catch constant or increasing before 1981 or 1986), as well as about the catch data set (beginning in 1981 or in 1986). All runs assumed that the variance of the CPUE estimates was constant within each CPUE series and among CPUE series. We chose to use equal weighting for all CPUE data points, since this was the base case weighting method used for the official stock assessment of sandbar sharks (Cortés et al. 2002). However, other weighting methods have also been considered, including giving zero weight to series that are considered to be less reliable, and interested readers could refer to Burgess et al. (2005) for a detailed description of the strengths and weaknesses associated with each CPUE series. Selectivity curves were specified to reflect the characteristics of the fishery to which each of the CPUE and catch data series correspond (Cortés et al. 2002; Apostolaki 2003). The commercial fishery targets mainly mature fish, whereas the recreational fishery targets smaller fish (ages 0–12).

Owing to lack of information about the virgin biomass of the population, a uniform prior probability density function (i.e., Uniform($500t$, 10^6t)) was used to describe the possible values of this parameter. The lower boundary was chosen to be of the same order of magnitude as the highest observed sandbar catches in biomass. The value of the upper boundary was chosen to be much greater than estimates of the mean virgin biomass for other large coastal sharks (Brooks et al. 2002; Cortés et al. 2002). The prior pdf assumed to represent the survival of pups at low population densities ($1/\alpha$; see eq. 4) is slightly informative, since it gives more probability to values close to 0.5 ($\beta(2,2)$). Truncated, normal prior pdfs were used for the historical (pre-1981 or pre-1986) commercial and recreational catches to ensure that simulated catches could take positive values only. The means of the simulated historical commercial and recreational catch prior distributions were set equal to the corresponding historic mean of the observed catches from 1981 to 2001 (number of fish): $C_{\text{comp,hist}} \sim N(70\,000, 40\,000^2)$ and $C_{\text{rec,hist}} \sim N(60\,000, 40\,000^2)$. The variance of each distribution was assumed to be similar to the variance in the corresponding historical catches. The constant of proportionality q and the log-normal standard deviation σ for residual errors between the observed and predicted values of relative fish abundance are also uncertain parameters. Noninformative prior pdfs were used for q and σ, and the integral of the likelihood function (see eq. 11) over q and σ was found analytically following Walters and Ludwig (1994).

Scenarios and management measures considered

To investigate the potential effects of alternative management measures on the future abundance of the sandbar shark population, the model was used to project the population into the future under management measures such as legal minimum length and the creation of marine protected areas. Deterministic projections and Bayesian decision analysis were used to evaluate the performance of the management measures.

The management measures considered in the simulations are as follows: closure of all commercial and recreational direct and indirect fishing (no exploitation); catch quota control only; catch quotas with legal minimum or maximum length; and catch quotas with the closure of specific areas to fishing (marine protected areas). The values of the uncertain parameters at the mode of the joint posterior pdf were used for the deterministic calculations. Three different age–size specific management measures were considered in conjunction with catch quotas: (*a*) legal minimum length under which recreational and commercial fisheries are not allowed to catch fish smaller than 110 cm total length (approximately 5 years old); (*b*) a quota on the number of mature fish that can be caught in the commercial fishery, but no quota on catches in the recreational fishery; and (*c*) an inverted slot limit to protect old juveniles and maturing fish, whereby fish aged 6–11 years cannot be exploited. The third measure

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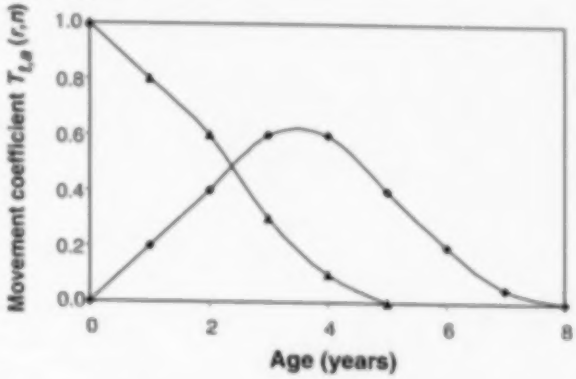
would affect the size distribution of the fish in the commercial as well as recreational catches, since both fisheries exploit maturing fish.

The model assumes that the annual catch quotas were unaffected by the introduction of the additional management measures. Although estimates of discard survival for sandbar shark are not available, there are some studies that provide estimates of discard survival for other shark species (Stevens et al. 2000*b*; PIRO 2005). Those studies suggest that discard survival might be high (above 50% for fish caught in longline) for at least some of the shark species (but see Borucinska et al. 2002). For simplicity, our model assumes that when a size limit or a size-based catch quota is in place, the survival of discarded sandbar sharks is equal to 1. However, interested readers could refer to Apostolaki (2005) for a detailed analysis of the sensitivity of model predictions about the effectiveness of fisheries management measures to changes in discard survival.

The total catch quota management measures were considered in combination with management measures that prohibit commercial and recreational fishing in portions of the managed areas. The simulation model made two different assumptions about the character of the marine protected areas: it assumed that fishing is prohibited in parts of the nursery areas of sandbar sharks or in parts of the secondary nursery areas of sandbar sharks. Nursery areas are areas occupied by young sandbar sharks (age < 4 years) during the summer. The simulation model assumes that young fish move to the nursery areas at the beginning of each summer and remain there for 3 months (one time step). Secondary nursery areas are areas occupied by older, immature sandbar sharks (sometime as old as 10 years) during the summer. The simulation model assumes that fish aged 1–7 years could move to secondary nursery areas each summer. These nursery and secondary nursery areas constitute less than 10% of the total area that sandbar sharks occupy (J. Carlson, National Marine Fisheries Service, Southeast Fisheries Science Center, Panama City Laboratory, 3500 Delwood Beach Road, Panama City, FL 32408, USA, personal communication; D. Grubbs, Hawaii Institute of Marine Biology, University of Hawaii, Post Office Box 1346, Kaneohe, HI 96744, USA, personal communication). Several areas used by immature sandbar sharks as nursery or secondary nursery areas have been identified in nearshore portions of the US Atlantic seaboard (Carlson (1999) and Merson and Pratt (2001) provide descriptions of these areas as well as of the studies undertaken in each area). The simulation model assumes that immature fish do not move between nursery areas or between secondary nursery areas over the summer. The model also assumes that fish that have moved to the marine protected area cannot be exploited during the 3-month period that they occupy the protected area but could be exploited at other times.

We used movement coefficients to describe the movement of fish to nursery or secondary nursery areas. This movement was assumed to take place at the beginning of summer. All fish that occupied the nursery or secondary nursery areas during the summer were assumed to move out of it at the end of summer. The model assumed that young fish did not move to the nursery or secondary nursery areas at any other period of the year. Thus, the values of the movement coefficient describe the movement of young fish to nursery or secondary nursery areas at the beginning of summer (Fig. 2). A description of the movement of young fish in quantitative terms is not available; therefore, the values of the transport coefficients shown in Fig. 2 are educated guesses based on qualitative descriptions of the migratory behavior of sandbar sharks (Merson and Pratt 2001; Grubbs et al. 2005).

Fig. 2. Fraction of the population in each age class a that is assumed to move to either a nursery or secondary nursery ground (area n) in the summer ($t = 3$): $T_{t,a}(r,n)$. Males and females are assumed to exhibit the same movement behavior. Triangles, movement to the nursery area; squares, movement to the secondary nursery area.



The protection of multiple nursery or secondary nursery areas can be simulated by varying the total size of the areas that are assumed to be protected. Our simulations assume that the size of the area protected by nursery or secondary nursery area closures varies from 10% to 100% of the total nursery or secondary nursery areas. Although time–area closures have been introduced off the US coast in the past few years to protect pups and juveniles sandbar sharks (e.g., the mid-Atlantic shark closure off of North Carolina) (NOAA 2003), the areas used in our simulations do not specifically represent any of the existing or proposed time–area closures.

Projections

For the deterministic calculations, the population was projected forward from the estimated status of the population in 2001 under the alternative management strategies. The values of the model parameters at the mode of the joint posterior pdf were used as input values of the model for the deterministic calculations. The parameter values at the mode of the joint posterior pdf for each run were the parameter values that maximize the product of the likelihood function (eq. 11) times the priors. The population size in 30 years (2031) was used as a performance measure for the deterministic projections. For the probabilistic projections, the following performance measures were calculated: (*a*) the probability that the number of fish in 10 years will be greater than 40% of the virgin population ($N_{tot(10)} > 0.4N_{tot(vir)}$); (*b*) the probability that the number of fish in 30 years will be greater than 40% of the virgin population ($N_{tot(30)} > 0.4N_{tot(vir)}$); (*c*) the probability that the number of fish in 30 years will be greater than 20% of the virgin population ($N_{tot(30)} > 0.2N_{tot(vir)}$); (*d*) the probability that the number of fish in 30 years will be greater than 60% of the virgin population ($N_{tot(30)} > 0.6N_{tot(vir)}$);

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Table 2. Model assumptions and input data used in the two reference runs.

	Run A	Run B
CPUE series	Age-independent and age-specific	Age-independent
Catch data	1981–2001	
Stock–recruitment function	Beverton–Holt	
Historical catches	Commercial, recreational	
Importance functions	Joint prior pdf	
Exploitation period	1974–2001	

Note: CPUE, catch per unit effort; pdf, probability density function.

and (*e*) the probability that the number of fish in 30 years will be less than 5% of the virgin population size (stock failure).

For each of the simulation runs considered, the probabilities described above (performance measures) were estimated using 5000 draws of the model parameter values taken from the discrete distribution of the weights in the resampling part of the SIR algorithm (Rubin 1987; Smith and Gelfand 1992). The time periods used for the probabilistic projections (10 and 30 years) were chosen to reflect the age at maturity (13 years) and maximum age (35 years) for sandbar shark (Table 1). We chose 40% of the virgin population size as a reference value, since studies on other fish species, including shark species, have indicated that depletion of a population to sizes smaller that 30%–40% of its virgin population size is indicative of overexploitation (Punt et al. 2000; Simpfendorfer et al. 2000; Hilborn 2002). The other two reference values correspond to 50% and 150% of that value.

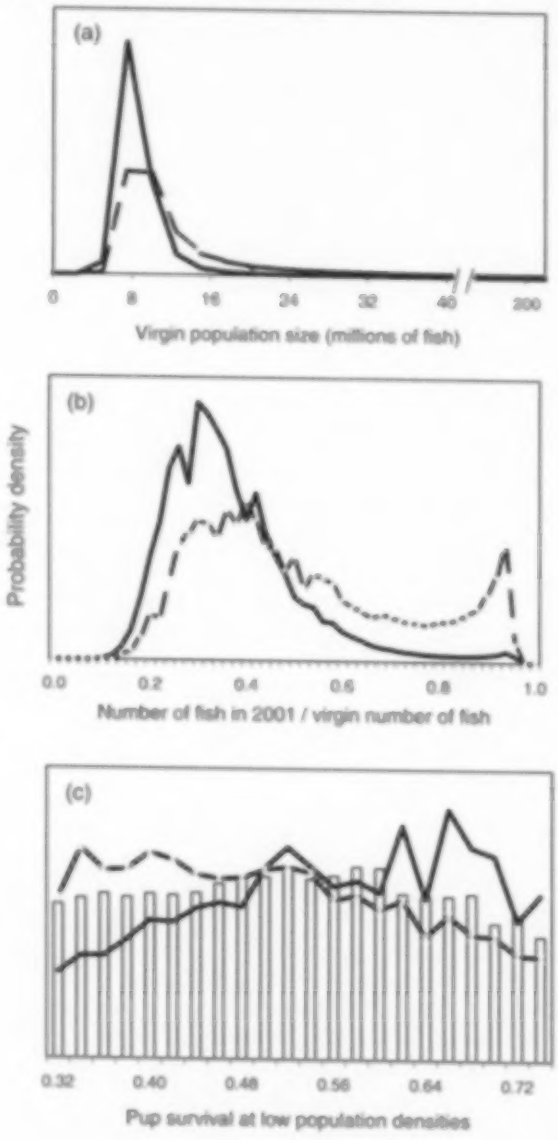
Results

Status of the population

The simulation runs conducted using different sets of assumptions (e.g., different sets of CPUE series and length of the catch series) can be separated into those that predict that the mean size of the population in 2001 was less than 40% of virgin number of fish (group A) and those that predict that the mean was at least 50% of virgin number of fish (group B) (Apostolaki 2003). None of the simulation runs provided empirical support for the mean size of the population in 2001 between about 40% and 50% of virgin population size. Because the results of the runs in each group were similar to each other, a run from the first group (run A) and a run from the second group (run B) were chosen for the projections. The specifications of the model for the two runs are shown (Table 2). The reference runs supported different levels of population depletion in 2001 and different population growth potential. Therefore, they can be used to explore the hypothetical outcomes of alternative management measures under different assumptions about the current status and the recovery potential of the stock.

Under both runs A and B, the marginal posterior pdf for the virgin size of the population in number of fish was skewed to the right, suggesting that the virgin size of the population was at the lower end of the range of values considered (Fig. 3*a*). Run B, though, assigned more probability

Fig. 3. Posterior probability density functions (pdf) for (*a*) population size under virgin conditions in number of fish, (*b*) ratio of current (2001) number of fish to virgin number of fish, and (*c*) pup survival at low population densities. Solid line, run A; broken line, run B. The prior pdf for the pup survival at low population densities is also shown (open bars).



to higher values of the population size under virgin conditions than did Run A. Run B also assigned more probability to higher values of the size of the population in 2001 than that predicted by Run A. This is reflected in the higher mean value for the size of the population in 2001 calculated under run B than that found under run A (Table 3). The depletion of the population in 2001 predicted under run B was also smaller than that found under run A (Fig. 3*b*, Table 3). With regard to pup survival at low population densities, the input

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Table 3. Values of the uncertain parameters at the mode of the joint posterior probability density function (pdf) and characteristics of the marginal posterior pdfs for the size of population under virgin conditions (number of fish and mature fish biomass), fish ratio, and pup survival for the two reference runs.

Parameter	Joint posterior pdf	Marginal posterior pdfs	
	Mode	Mean	95% PI
Run A			
Number of fish (millions)	7.0	9.7	6.6, 11.5
Mature fish biomass (×1000 t)	60	84	57, 130
Pup survival	0.74	0.55	0.35, 0.72
Fish abundance ratio	0.32	0.39	0.23, 0.65
Run B			
Number of fish (millions)	8.5	20	6.9, 96
Mature fish biomass (×1000 t)	73	170	59, 830
Pup survival	0.50	0.51	0.32, 0.73
Fish abundance ratio	0.36	0.55	0.25, 0.95

Note: Fish abundance ratio is the ratio of the fish number in 2001 to the assumed virgin fish number. Pup survival is the pup survival at low population densities. PI is the probability interval.

Table 4. Model predictions about sustainable exploitation levels for reference runs A and B assuming that the ratio of commercial and recreational exploitation rate is equal to the corresponding ratio in 2001 (exploitation rate ratio (H_{comm}/H_{rec}) = 4).

Parameter	Run A		Run B	
	Mean	CV	Mean	CV
MSY (t)[a]	780	0.86	1400	1.42
Exploitation rate at MSY	0.019	0.35	0.016	0.41
Relative stock size[b] at MSY	0.40 (0.45)	0.17	0.38	0.23
MSY/B_{vir}[c]	0.0063 (0.0085)	0.40	0.0054	0.50

Note: The estimated mode of the parameters under run A is also shown in a parenthesis next to the mean value where the mode differs from the mean. CV, coefficient of variation.

[a]Maximum sustainable yield.

[b]Number of fish.

[c]B_{vir}, population biomass under virgin conditions.

data were only slightly informative (Fig. 3c). However, the posterior pdf from run A assigned higher credibility to those values of pup survival at low population density that were close to 0.7 than was assigned by the prior pdf. On the other hand, the posterior for pup survival calculated under run B assigned maximum probability to lower values of survival (Fig. 3c, Table 3). The CPUE data were uninformative about the historical exploitation; therefore, the marginal posterior pdf for the historical commercial and recreational catches were the same as their priors.

The maximum sustainable yield (MSY) was calculated under each run assuming that the ratio of the commercial to the recreational exploitation rate was the same as the corresponding ratio in 2001, which was equal to four. Although the commercial and recreational catches in 2001 were similar, the commercial exploitation rate in 2001 was greater than the recreational exploitation rate in the same year. This is because the commercial sector targets older fish, the number of which is generally smaller than the number of fish of younger age (see relevant discussion in Data and Bayesian priors section). Therefore, the commercial exploitation rate that corresponds to given catches in number of fish is higher than the recreational rate that would result in the same catches. The calculations under run A resulted in much smaller estimates of MSY than those calculated under run B (Table 4). The main reason for this is that under run B, the predictions of the model for the population biomass under virgin conditions (B_{vir}) were much higher than those found under run A (Table 3), whereas the model predictions about the exploitation rate that leads to MSY remained almost the same for both cases (Table 4). Thus, for the same exploitation effort, the MSY calculated under run B would be higher than that under run A. Nevertheless, the predictions of the model about the mean value of the relative size of population at MSY (N_{MSY}/N_{vir}) and the maximum biomass that can be extracted sustainably relative to the virgin biomass (MSY/B_{vir}) was almost the same for both runs.

The predictions of the model about the virgin population, current population, and MSY are characterized by high uncertainty. This is mainly the result of the paucity of information in the catch and CPUE data as well as in the prior pdfs used. Under most of the scenarios considered, the model provided more precise predictions about the depletion of the population (relative values) rather than its absolute magnitude. The model also gave more precise estimates of the effort at MSY, relative size of the population at MSY, and fraction of the population that could be removed annually relative to the virgin population. The results showed that the

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Table 5. Decision analysis evaluating stock size as a performance indicator against several biological reference points for reference runs A and B and two levels of exploitation: catch quotas equal to the catch quotas in numbers in 2001 (status quo) and catch quotas equal to zero (no fishing).

	Probability of meeting stock condition			
	Run A		Run B	
Stock condition	Status quo	No fishing	Status quo	No fishing
$N_{tot(10)} > 0.4N_{tot(virg)}$	0.12	0.48	0.43	0.70
$N_{tot(30)} > 0.4N_{tot(virg)}$	0.05	0.76	0.29	0.86
$N_{tot(30)} > 0.2N_{tot(virg)}$	0.14	1.00	0.46	1.00
$N_{tot(30)} > 0.6N_{tot(virg)}$	0.02	0.15	0.18	0.42
Stock failure$_{(30)}$	0.67	0.00	0.33	0.00

Note: $N_{tot,z}$, total number of fish after the population has been projected z years into the future; $N_{tot(virg)}$, total number of fish under virgin conditions; stock failure$_{(30)}$, probability that the population size will become less than 5% of virgin size in the next 30 years (starting in 2002). See Table 6 for a description of the results in qualitative terms.

Table 6. Qualitative predictions about the status of the stock under levels of exploitation described in Table 5 (also refer to Fig. 4).

Status quo	No fishing
Decision analysis	
High probability of stock failure under run A; moderate probability of stock failure under run B	Population recovery More optimistic results about the future status of the stock under run B than run A
Deterministic calculations	
Stock failure predicted under both runs Stock failure happens more quickly under run A than run B	Population recovery Greater recovery rate under run A than run B The size of the stock in 30 years is greater under run A than run B

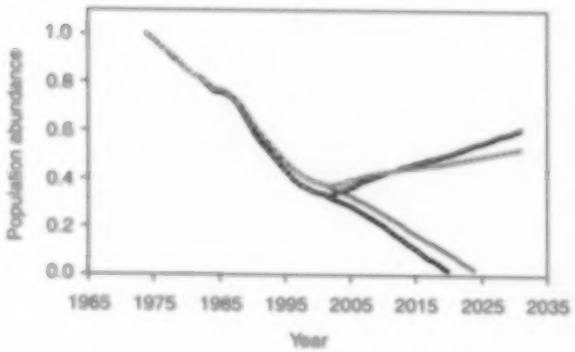
removal of a small portion of the population (equal to or less than one-hundredth of the virgin biomass) is enough to lead to more than a 50% reduction in the population size.

Projections

Recovery potential

The ability of the population to recover was evaluated by assuming a comprehensive and effective fishery closure and observing the changes in the size of the population over time. According to the deterministic results, the population recovered more quickly under simulations based on the mode of the parameter values estimated in run A and more slowly under simulations based on the mode of parameter values estimated in run B (Fig. 4). The reason for that was that the mode of pup survival at low densities under run A was much higher than that estimated under run B (Fig. 3c). Therefore, although run A gave more pessimistic results about the current status of the stock than did run B, it gave more optimistic results about the ability of the population to recover. The results of the probabilistic decision analysis showed that under the no-fishing assumption, it was almost certain that the population abundance in 30 years would exceed 20% of the virgin abundance regardless of the values of the uncertain parameters (Tables 5 and 6). However, for both runs, there was less than 50% chance that the population would be

Fig. 4. Population abundance (number of fish) under two exploitation regimes: (*i*) no fishing after 2001 (solid line with open circles) and (*ii*) future catches in number of fish are equal to the catches in 2001 (solid line). Reference run A is shown with a black line; reference run B is shown with a grey line. Population abundance is normalized to the population abundance under virgin conditions. For comparison, the size of the population prior to 2002 under runs A and B is also shown (solid line with open circles).



above 60% of its virgin abundance in 30 years. In contrast with the results of the deterministic calculations, the results of the probabilistic calculations about the future status of the population were more optimistic under simulations based on model parameter values estimated in run B than under simulations based on model parameter values estimated in run A. This is due to the fact that the probabilistic analysis yielded more optimistic results about the current status of the stock under run B than under run A, whereas mean pup survival predictions were similar for both model runs (Table 3). Projections that assumed that future catches were the same as the catches in 2001 indicated that such catches are unlikely to be sustainable (Table 5). This prediction was true under both runs and regardless of whether uncertainty was taken into account.

Reference future catches

To further examine the differences between deterministic and probabilistic analyses, the consequences of alternative management approaches were compared under deterministic and probabilistic decision analysis frameworks, for a total

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Fig. 5. Population abundance projections for reference runs A (*a*) and B (*b*) when future catch quotas (numbers) for the commercial fishery are one-quarter of those in 2001, and future catch quotas for the recreational fishery are one-half of those in 2001. The results are given for the values of the uncertain parameters at the joint posterior pdf mode. Population abundance is normalized to population abundance under virgin conditions. Solid line, catch quota reduction (CQR) only; open circles, CQR and legal minimum length; asterisks, CQR and limitations in the number of mature fish caught. The line that corresponds to the model predictions when CQR is combined with protection of young juveniles and subadults would be between the solid line and the asterisks; however, for clearness of the graph, that line has been omitted. The solid line also indicates when CQR is applied together with nursery or secondary nursery areas protection.

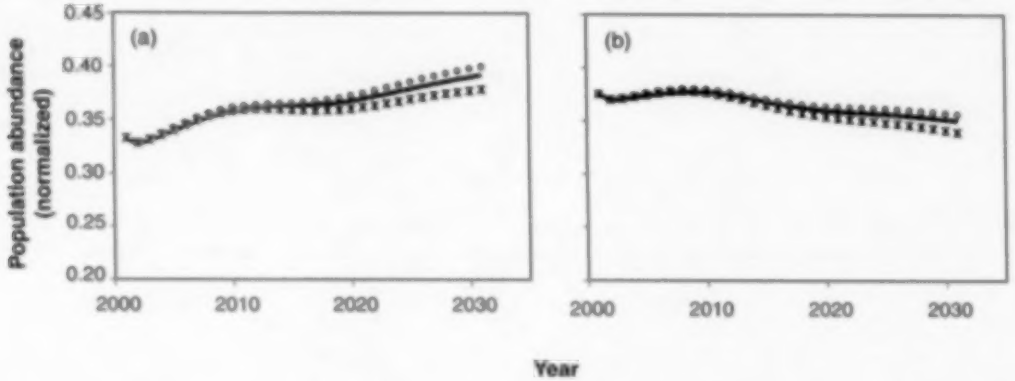

Table 7. Decision analysis evaluating stock size as a performance indicator against several biological reference points for reference runs A and B, assuming that the future catch quotas for the commercial fishery are one-quarter of that in 2001 and the future catch quotas for the recreational fishery are one-half of the recreational catch quotas in 2001.

	Probability of meeting stock condition	
Stock condition	Run A	Run B
$N_{tot,30} > 0.4N_{tot,virg}$	0.28	0.60
$N_{tot,30} > 0.4N_{tot,virg}$	0.32	0.60
$N_{tot,30} > 0.2N_{tot,virg}$	0.84	0.92
$N_{tot,30} > 0.6N_{tot,virg}$	0.05	0.28
Stock failure$_{(30)}$	0.00	0.00

Note: $N_{tot,z}$, total number of fish after the population has been projected z years into the future; $N_{tot,virg}$, total number of fish under virgin conditions; stock failure$_{(30)}$, probability that the population size will become less than 5% of virgin size in the next 30 years (starting in 2002).

catch quota that was potentially sustainable. Given that initial investigations suggest that there is high probability that the population will decline if the catches continue at 2001 levels, smaller future catch quotas were considered. The level of exploitation was chosen such that based on the results of the deterministic predictions, it would not cause further decreases in population size. Since the deterministic results of the two runs were different, the catch quotas were chosen such that under the most pessimistic scenario (run B), the population would remain constant in the future. The catch quotas chosen for the recreational fishery were one-half of the recreational catch quotas in 2001. The future commercial catches were assumed to be equal to one-fourth of the corresponding catch quotas in 2001. For run A, the deterministic calculations showed that for the chosen catch levels, the population would increase in the future (Fig. 5).

The predictions of the decision analysis also showed that the reduction in the catch quotas would result in the population being constant or increase in the future (Table 7). For runs A and B, the adoption of smaller catch quotas reduced the likelihood of stock failure (Table 7). The probabilistic calculations also showed that the population under run B would remain constant and close to the population size that could support MSY, whereas the population under run A would probably remain below the population size that could support MSY.

Alternative management measures

The effects of combining the reference catch quotas with a legal minimum length, limitations in the number of mature fish that are caught per year, a size limit to protect old juveniles and maturing fish, protection of nursery areas, and protection of secondary nursery areas were also evaluated. The deterministic and probabilistic analyses gave different results about the relative effectiveness of these management measures (see Table 8 for the ranking of management measures).

When the reference catch quotas were combined with a legal minimum length, the results of the decision analysis about the future status of the stock were much more pessimistic than the predictions when only the catch quotas were applied (Tables 7 and 9). The exclusion of young fish from the exploitable population resulted in an increase in fishing pressure on maturing fish and adults. The decline in population growth due to the removal of a greater number of older fish than those taken without a legal minimum length was more substantial than the positive contribution accruing from the protection of young fish, and thus it led to the more pessimistic predicted outcomes. On the other hand, according to the results of the deterministic projections, the future size of the population when catch quotas were combined with a legal minimum length was marginally greater than the size of

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Table 8. Qualitative predictions about the performance of different management scenarios based on the results of stochastic calculations (decision analysis) or deterministic calculations.

	Catch quotas	Legal minimum length	Protection of mature fish	Protection of juveniles and maturing fish	Protection of nursery areas	Protection of secondary nursery areas
Decision analysis	1*	4	2	3	1*	1*
Deterministic calculations	2	1*	4	3	2	2

Note: Catch quotas for the commercial fishery are assumed to be one-quarter of the commercial catch quotas in 2001; catch quotas for the recreational fishery are assumed to be one-half of the recreational catch quotas in 2001. All the other management measures are assumed to be applied in combination with the catch quotas. The management scenarios with the best performance with regards to the status of the stock is represented by 1*; the least effective management scenario (worst performance) is shown by 4. Table 9 gives the quantitative results of the calculations.

Table 9. Decision analysis evaluating stock size as a performance indicator against several biological reference points for reference runs A and B, assuming that catch quotas for the commercial fishery are one-quarter of the commercial catch quotas in 2001 and catch quotas for the recreational fishery are one-half of the recreational catch quotas in 2001.

	Probability of meeting stock condition			
Stock condition	Legal minimum length	Maximum no. of mature fish caught	Protection of juveniles and maturing fish	Protection of nursery areas
Run A				
$N_{tot(10)} > 0.4N_{tot(virg)}$	0.12	0.27	0.20	0.28
$N_{tot(20)} > 0.4N_{tot(virg)}$	0.15	0.29	0.22	0.32
$N_{tot(30)} > 0.2N_{tot(virg)}$	0.43	0.82	0.62	0.84
$N_{tot(30)} > 0.6N_{tot(virg)}$	0.04	0.05	0.05	0.05
Stock failure$_{(30)}$	0.22	0.00	0.06	0.00
Run B				
$N_{tot(10)} > 0.4N_{tot(virg)}$	0.43	0.60	0.53	0.60
$N_{tot(20)} > 0.4N_{tot(virg)}$	0.45	0.59	0.53	0.60
$N_{tot(30)} > 0.2N_{tot(virg)}$	0.72	0.91	0.82	0.93
$N_{tot(30)} > 0.6N_{tot(virg)}$	0.24	0.27	0.26	0.28
Stock failure$_{(30)}$	0.07	0.00	0.01	0.00

Note: The catch quotas are applied in combination with minimum or maximum size limit or area closures. The second row of the table (except first column) shows the management measures that are applied in combination with the catch quotas. The results when we assume that the secondary nursery areas are protected are the same as those when the nursery areas are protected. $N_{tot,y}$, total number of fish after the population has been projected y years into the future; $N_{tot(virg)}$, total number of fish under virgin conditions; stock failure$_{(30)}$, probability that the population sizes will become less than 5% of virgin size in the next 30 years (starting in 2002).

the population when the same catch quotas were used without any other measure (Fig. 5). These results imply that for the state of nature that corresponds to the mode of the posterior distribution, any negative effects on population growth due to the increase in fishing pressure on old fish were counterbalanced by the protection of young fish. However, in many of the states of nature considered in the decision analysis, this small increase in the fishing pressure on maturing fish and adults was enough to lead to further decline of the population size.

The probabilistic simulations suggest that protection of mature fish or of juveniles and maturing fish would lead to predicted outcomes that were closer to but still more pessimistic than the results found when only total (not size-specific) catch quotas were used (Table 9). The results when the number of mature fish that could be caught was regulated were the least pessimistic of the results taken under any of the three alternative size-limit management strategies in the probabilistic decision analysis. In the deterministic analysis, the protection of old juveniles gave the same results as those found when only catch quotas were used for the management of the fishery. When a combination of catch quotas and limitation in the number of mature fish that could be caught annually was used, the results of the deterministic calculations showed that this combination was the least effective of all (Fig. 5). According to the results of the decision analysis, this combination would be more effective than the combination of catch quotas with any of the other two management measures (Tables 8 and 9). For example, the probability that the population is above 40% of virgin size in 30 years is equal to 0.29 when catch quotas are combined with limitation in the number of mature fish that could be caught annually. This probability reduces to 0.22 and 0.15 when catch quotas are combined with protection of juveniles

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– maturing fish or with legal minimum length, respectively (Table 9). Similarly, the probability of stock failure is much smaller when catch quotas are combined with limitation in the number of mature fish that could be caught annually than when catch quotas are combined with any of the other two measures in the probabilistic analysis.

When a closed area was used to protect part of the nursery area and, therefore, a fraction of the immature fish, the predictions of the probabilistic calculations were the same as the model predictions when only catch quotas were applied. In this case, the fishing pressure on the age classes that were not protected did not increase as much as when a legal minimum length was adopted. The fish were protected only for part of the year and could be exploited once they had moved out of the marine protected area. Because of that, the changes in the size distribution of the population that could be exploited were not as great as when a legal minimum length was applied. Thus, relative to the results found when only catch quotas were used, the introduction of a closed area did not affect the model predictions negatively (Table 7 and last column in Table 9) as happened, for example, with the legal minimum length. However, any increase in biomass or survival of fish in the age groups that were partly protected was still counterbalanced by the higher exploitation of the fish in the other age classes. This was also true when part of the secondary nursery area was protected. For both runs A and B, the deterministic projections also gave results that were the same as the results found when only catch quotas were used (Fig. 5). The results were the same regardless of whether the marine protected area constituted a nursery or secondary nursery area. The results were also insensitive to changes in the proportion of the nursery or secondary nursery area that was protected, because the marine protected area did not reduce the overall fishing mortality. For this reason, the quantitative results for only the case in which the whole nursery area is protected are presented (last column in Table 9).

Overall, although a legal minimum length combined with a total catch quota was the most effective strategy according to the deterministic projections, under uncertainty, none of the management alternatives considered performed better than the catch quota reduction alone (Tables 7 and 9 (quantitative results) and Table 8 (ranking of the strategies)).

Discussion

Several studies have focused on the potential of managing a fishery using reference points, such as $F_{0.1}$, F_{MSY}, and MSY, in the light of uncertainty (Mace 1994; Hilborn et al. 2002; Prager et al. 2003). Those studies have covered, in depth, the benefits and disadvantages related to reference points, utilization of catch, or fishing effort quotas and their robustness to uncertainty. This study considered a range of additional measures and examined how the use of probabilistic decision analysis versus deterministic projections affected the apparent effectiveness of alternative fishery management strategies and whether unequivocally robust measures for the sustainable exploitation of a species could be found despite considerable levels of uncertainty.

Up to now, simple population dynamics models, such as demographic analysis and surplus production models largely structured as deterministic analyses, have been used for the simulation of the dynamics of sandbar shark populations and also to assess stock status and response to exploitation pressure (Smith et al. 1998; Brewster-Geisz and Miller 2000; but see Cortés et al. 2002). Here, a detailed, age-structured population dynamics model was utilized to increase the realism in the simulation of sandbar shark dynamics and facilitate the evaluation of the potential of management measures, such as size-specific limits, which cannot be simulated with simpler models. In addition, the use of probabilistic decision analysis facilitated evaluation of the potential consequences of alternative management measures while taking into account parameter and structural uncertainty.

Implications for shark fishery management

The analysis showed that catch levels similar to those in 2001 are unlikely to be sustainable for sandbar shark in the western part of the North Atlantic. The simulations suggest that the sandbar shark population can only sustain low levels of exploitation and that the population will recover slowly once it has been depleted. The results of the analysis supported values for the relative stock size at MSY, which were close to 40% of the estimated virgin stock. These values are higher than the reference points that are used for many commercial fish species (Richards and Maguire 1998; Mace 2001; Hilborn 2002) but are not unexpected given the low fecundity and late maturation of sandbar sharks. Similarly, the fraction of population biomass that could be harvested annually relative to the virgin population biomass without causing a reduction in population size to levels smaller than those at MSY is close to 0.01. This fraction is similar to the corresponding values found by Punt and Walker (1998) for school shark off southern Australia (<0.02), but it is considerably smaller than that predicted for many other commercial fish species. For example, the value of this fraction for Atlantic yellowfin tuna (*Thunnus albacares*) is near 0.2 (ICCAT 2004), whereas for swordfish (*Xiphias gladius*) in the North Atlantic, this fraction ranges from 0.05 to 0.2 (ICCAT 2003). The value of this fraction for sandbar shark appears to be closer to the values considered sustainable for marine mammals (Lankester and Beddington 1986; Johnston et al. 2000). Such predictions highlight the need to tailor fishery management based on species-specific predictions. This is particularly important when management plans are developed for multispecies fisheries that target fish species that exhibit different levels of resilience to exploitation.

Cortés (2002) suggested that the protection of juveniles and adult sharks is more important than the protection of age-0 individuals. In particular, for sandbar shark, Cortés concluded that juvenile survival is the most important factor for the increase in population growth. The results presented here (probabilistic calculations) also indicate that the protection of old juveniles and adults might be more important for the recovery of the population than the protection of younger fish. However, our analysis shows that the protection of adults has greater probability to assist the recovery of the population than the protection of old juveniles and maturing fish. Under the assumptions considered here, any change in the survival of specific age groups due to changes in fishing practices automatically affects the survival of the remaining age groups (i.e., decreased fishing pressure on adults would

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result in increased mortality of younger fish). A possible reason for why our results differ from Cortés (2002) is that Cortés' model does not allow for effort to be redistributed to unprotected age classes.

Closed areas have been considered in the past as a plausible management measure for the protection of specific shark populations (Carrier and Pratt 1998; NMFS 2001; Apostolaki et al. 2002). However, the effects of establishing closed areas to prevent the exploitation of shark populations have not been studied in depth. Here, the potential effects of management options that utilize closed areas to protect part of the population were quantified. The results suggest that the protection of nursery or secondary nursery areas would not likely increase the resilience of the population to overexploitation.

Baum et al. (2003) estimated the potential effects of closed areas on the population of various shark species using relative abundance information provided by fisheries that catch sharks as bycatch. They found that closure of specific inshore areas could provide conservation benefits to some shark populations. They concluded that the establishment of marine protected areas could protect populations that reside in the protected areas but could have negative effects on the population of other species that live outside the closed area because of reallocation of the fishing pressure. Our study suggests that the establishment of a marine protected area might not be appropriate for the management of fisheries that impact shark populations spending only part of the year in the protected area; Baum et al. (2003) did not account for movement of fish into and out of the protected area. The differences in the results of these two studies indicate that realistic simulation of movement of highly migratory species may exert an important influence on model predictions. Consequently, migratory behavior needs to be taken into account when model-based advice is provided. Punt et al. (2000) also supports this conclusion by showing that the inclusion of spatial structure in assessment models could markedly reduce uncertainty in model predictions.

Measures such as legal minimum length, spatial protection of areas of major importance, and protection of the spawning stock seek to increase the growth of the population by either increasing the yield per recruit or the spawners per recruit. Because of the biological characteristics of the sandbar shark (low growth rate, small number of pups, late age at maturity), the simulation model suggests that these measures would be unlikely to mitigate the effects of overexploitation or contribute to stock recovery. With regard to the three size-based management measures considered (legal minimum length and size limits, which control the retention of juveniles and maturing fish or the retention of adults), the results of the analyses indicate that for a given total catch, the exclusion of any of the age groups (adult, juveniles, etc.) from the exploitable population could negatively affect the status of the stock. These measures are expected to perform even more poorly than our model predicted if the discard survival is smaller than one (the value used in our analysis).

Deterministic versus probabilistic results and adoption of robust management measures

Our analysis showed that inference about the recovery potential of the population and its sensitivity to exploitation is possible despite high uncertainty in the predictions of the model about the virgin and current stock size. This can be achieved by expressing key parameters in relative terms. For example, although the model predictions for MSY were highly imprecise, the predictions for the stock size at MSY relative to the virgin stock size as well as the MSY relative to the virgin stock size were estimated with higher precision. Such predictions combined with information about key biological characteristics could provide an insight into the level and type of exploitation that a population can sustain and therefore help in the adoption of potentially effective management measures.

The fact that none of the alternative management strategies considered in the analysis performed better than the use of catch quotas alone indicates that in the light of considerable uncertainty about the status of a stock, the distribution of the fishing effort to all age groups of the population is probably a more risk-averse management approach than targeting specific groups such as juveniles or adults without further reduction in the fishing mortality. Thus, our findings provide an insight into the biological characteristics of species that may not benefit from the use of management measures for the protection of specific age groups.

The results of our analysis indicate that predictions about the effectiveness of alternative management strategies for fisheries depend on whether the policies are evaluated under deterministic or probabilistic decision analyses. Management approaches that under the deterministic projections appeared to be most effective actually failed to provide the highest benefits when uncertainty was taken into account. Tanner (2003) compared the deterministic predictions of his model about the effectiveness of a chosen fishing strategy for prawn fishery with the predictions of the model when uncertainty in the parameter values was taken into account. He concluded that the qualitative predictions of the model remained the same regardless of whether deterministic or probabilistic calculations were employed. This was also true for some of the management approaches tested in our analysis. However, we found that the effectiveness of management approaches, such as the use of catch quotas together with protection of specific age groups, was sensitive to the inclusion of uncertainty in the calculations. These results are in agreement with the predictions of Hilborn et al. (2002), which indicated that it is important to evaluate the effects of management measures on a case-by-case basis, taking into account the life history parameters of the stock and the uncertainty in the system.

Although different factors that affect the precision of the predictions have been considered, some sources of uncertainty (i.e., environmental variability) have been ignored. We also examined the effectiveness of alternative management strategies using biological reference points – performance indicators (e.g., probability of stock collapse, probability of the population size being above 40% the virgin size), but we did not consider the economic effects of the alternative management strategies. Stefansson and Rosenberg (2005) conducted an analysis on the effectiveness of alternative fisheries management regimes using economic returns as one of the performance measures. They found that model predictions regarding the more effective management strategies differed depending on whether uncertainty was incorporated in the

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analysis or not, a conclusion which is consistent with our findings.

Our study shows that it is possible to provide advice for the management of fisheries in the light of considerable uncertainty in the system. By comparing alternative fishery management strategies in a probabilistic context (decision analysis), we also provide indications of management measures that could be effective under a range of biological and exploitation conditions. Our study also suggests that the results of deterministic policy evaluations could be misleading and shows that increasing the level of realism in the simulation of the system of interest could enable a better understanding of the likely effects of different management measures, thus assisting in the adoption of more effective fishery management strategies.

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A diatom-based index for the biological assessment of eastern Canadian rivers: an application of correspondence analysis (CA)

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Abstract: We developed a diatom-based index that integrates the effects of multiple stresses on streams and provides information related to the "distance" from the nonimpacted state. The Eastern Canadian Diatom Index (IDEC) was based on a correspondence analysis (CA) to develop a chemistry-free index where the position of the sites along the gradient of maximum variance (first axis) is strictly determined by diatom community structure and is therefore independent of measured environmental variables. The index value indicates the distance of each diatom community from its specific reference community. A high index value represents a non- or less-impacted site, while a low index value represents a more heavily impacted site. Two sub-indices were developed based on two sets of reference communities. The IDEC-circumneutral includes the sites that have reference communities characteristic of slightly acidic or neutral environments. The IDEC-alkaline includes the sites that have reference communities characteristic of environments where pH values are naturally higher than 7.5. The distinction between the two sub-indices is fundamental to make sure that each stream has the potential to reach a high IDEC value following complete restoration of its ecosystem.

Résumé : Nous avons mis au point un indice basé sur les diatomées qui intègre les effets des nombreux stress sur les cours d'eau et qui fournit des renseignements sur la « distance » avec une état non perturbé. L'Indice Diatomées de l'Est du Canada (IDEC) est construit à partir d'une analyse des correspondances (AC) de manière à ce que la position des sites sur le gradient de variance maximale (premier axe) soit uniquement déterminée à partir de la structure des communautés de diatomées et qu'elle soit indépendante des variables environnementales. L'échelle de l'indice indique la distance entre une communauté de diatomées et sa communauté de référence spécifique. Une valeur élevée de l'indice indique une bonne intégrité écologique alors qu'une valeur faible est associée à une communauté perturbée. Deux sous-indices furent développés à partir de deux séries de communautés de référence. L'IDEC circumneutre inclut les communautés de référence des milieux légèrement acides ou neutres. L'IDEC alcalin, pour sa part, inclut les communautés de référence des milieux ayant un pH qui est naturellement supérieur à 7,5. La distinction entre les deux sous-indices est fondamentale, puisqu'elle assure que toute rivière ait le potentiel d'atteindre des valeurs indicielles élevées, advenant la restauration de son écosystème.

Introduction

Physico-chemical measurements used in water quality monitoring do not provide a temporally integrated picture of the response of ecosystems to external stresses, nor do they provide information about the effects of these changes on biological communities. For example, (*i*) nutrient concentrations do not necessarily reflect the quantity that is bioavailable (Winter and Duthie 2000), (*ii*) high growth rates may deplete ambient nutrient concentrations resulting in a low concentration of nutrients remaining in the water column at the time of sampling (Biggs 1990), (*iii*) based on traditional measurements, a difference in phosphorus concentration can be interpreted as being equivalent when observed in the lower range or the higher range, while biological indicators have the ability to show that these differences may not be ecologically comparable. The use of bioindicators provides an integrated measurement of water quality as experienced by the aquatic biota and offers a useful addition to chemical-based water quality.

Several recent studies carried out in Canada and United States show the potential of diatom communities as indicators of water quality (e.g., Hill et al. 2000; Winter and Duthie 2000; Potapova and Charles 2002). Despite the fact that dia-

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toms have proven to be good indicators of environmental conditions, there are no such indices currently used in biomonitoring programs in Canada. European countries have a long history in developing diatom-based indices for biological assessment and biocriteria. A variety of indices has been developed, and among the most popular are the Specific Polluo-sensitivity Index (IPS) (Coste 1982), the Diatom Biological Index (IBD) (Lenoir and Coste 1996; Prygiel and Coste 2000), the Trophic Diatom Index (TDI) (Kelly and Whitton 1995), and the Sladecek Index (SLA) (Sladecek 1973). All of the above indices were developed based on a weighted average equation (Zelinka and Marvan 1961), where the optima and tolerance values for each taxon need to be determined. The differences between these indices are related to the number of taxa (genus, species, varieties) that were used to develop the indices, the sensitivity value (optima), and indicator value (tolerance) that were attributed to each taxon, as well as the water quality information provided (e.g., general index, trophic index, organic pollution index). European indices have been used to assess water quality in numerous countries and proved to be useful (e.g., Eloranta and Andersson 1998; Kwandrans et al. 1998). However, a study evaluating the potential use of European indices in Canada (Campeau et al. 2005) revealed the following limitations: (*i*) European indices do not include all the taxa that were observed in Canadian streams, (*ii*) the ecological optimum for certain taxa seems to differ between Europe and Canada, (*iii*) the most impacted sites in Canada were rarely attributed the worst water quality values, indicating that the level of alteration is lower for Canadian streams than for European streams, and (*iv*) some European indices do not properly identify Canadian reference conditions, resulting in index values that do not reflect non- or less-impacted communities. Furthermore, species optima and tolerances are generally derived from physico-chemical data, which implies that biomonitoring depends on environmental variables, leading to a circular argument. The main interest for using biota is precisely the complementary and nonredundant information provided on the ecosystem status. The most logical approach, in terms of ecological integrity, is to derive bioindication directly from community structure.

The goal of the project was to develop a diatom-based index that integrates different types of stream alterations and provides information related to the "distance" (i.e., the position of the sites along the gradient of maximum variance (first axis)) from the least-impacted state. The Eastern Canadian Diatom Index (IDEC, Indice Diatomées de l'Est du Canada) developed in this study is based on a correspondence analysis (CA) for the interpretation of the biological distances (measured as differences in diatoms communities) between sites. A CA is a series of weighted averaging operations allowing the ordination of samples according to species ecology (i.e., that the response of the species along an environmental gradient is derived from their optima and tolerances). Weighted averaging ordinations have the advantage of ordering sites and species simultaneously along the gradients of maximum variance. Ordinations allow reduction in the number of dimensions with minimum loss of information; similar communities are grouped together and dissimilar communities are far apart. An advantage of using CA to develop a diatom-based index for Canadian streams is that the autecology of each taxon does not need prior investigation, which is a limitation with most of the existing indices pointed out by Passy and Bode (2004) and Wunsam et al. (2002).

The index proposed in the present study is entirely based on ordinations and provides the positions of sites along the gradient of maximum variance (agricultural pollution). The IDEC was based on CAs instead of a canonical correspondence analysis (CCA) to develop a chemistry-free index, where the position of the sites along the gradient of maximum variance (first axis) is strictly determined by diatom community structure and therefore is independent of measured environmental variables.

Materials and methods

Study area

The streams were selected in the southern part of Quebec (Canada) so that a wide range of watershed characteristics was included (Fig. 1). The samples were collected at 126 sampling locations distributed along 32 streams and creeks in the St. Lawrence River Basin. The sampling sites were distributed within three ecoregions: the Canadian Shield, the St. Lawrence Lowlands, and the Appalachians. The Canadian Shield catchments are mostly covered by boreal forest, but the southern part of the Shield overlaps the transition zone of mixed and boreal coniferous forests. The streams sampled are low in nutrients, conductivity, and suspended solids and exhibit circumneutral pH (Table 1). These catchments are considered less impacted. The St. Lawrence Lowlands are characterized by intensive farmlands, large industrial centers, and are the location of most of Quebec's population. The streams sampled are high in nutrients, conductivity, and suspended solids and have higher pH (Table 1). These catchments exhibit a gradient from slightly impacted to very impacted streams, most of the latter being located in the Upper St. Lawrence Plain. Located in southeastern Canada, the catchments located in the Appalachians are also impacted by farming, but to a lesser extent. The streams sampled have intermediate levels of nutrients, conductivity, and suspended solids. Many streams have their source in the Canadian Shield or the Appalachians and flow downstream through the St. Lawrence Lowlands. As a result, the water chemistry of some streams flowing through the lowlands reflects the characteristics of the ecoregion upstream.

Data collection

Most water samples were collected on a 4-week basis and analyzed by the Ministry of the Environment of Quebec. The following parameters were considered in this study: total phosphorus (TP), total nitrogen (TN), nitrogen from ammonia (NH_3-N), chlorophyll *a*, pH, conductivity (CON), temperature (TEMP), dissolved oxygen (O_2), turbidity (TUR), suspended solids, coliforms (CF), and dissolved organic carbon (DOC). Latitude, longitude, altitude, geology, distance to source, slope, catchment area, river morphology, and land use were determined using geographic information systems (GIS) at the Université du Québec à Trois-Rivières (Trois-Rivières, Quebec). Stream width, current velocity, riparian zone characteristics, and stream bed composition were evaluated on site. Because diatoms integrate stream water chem-

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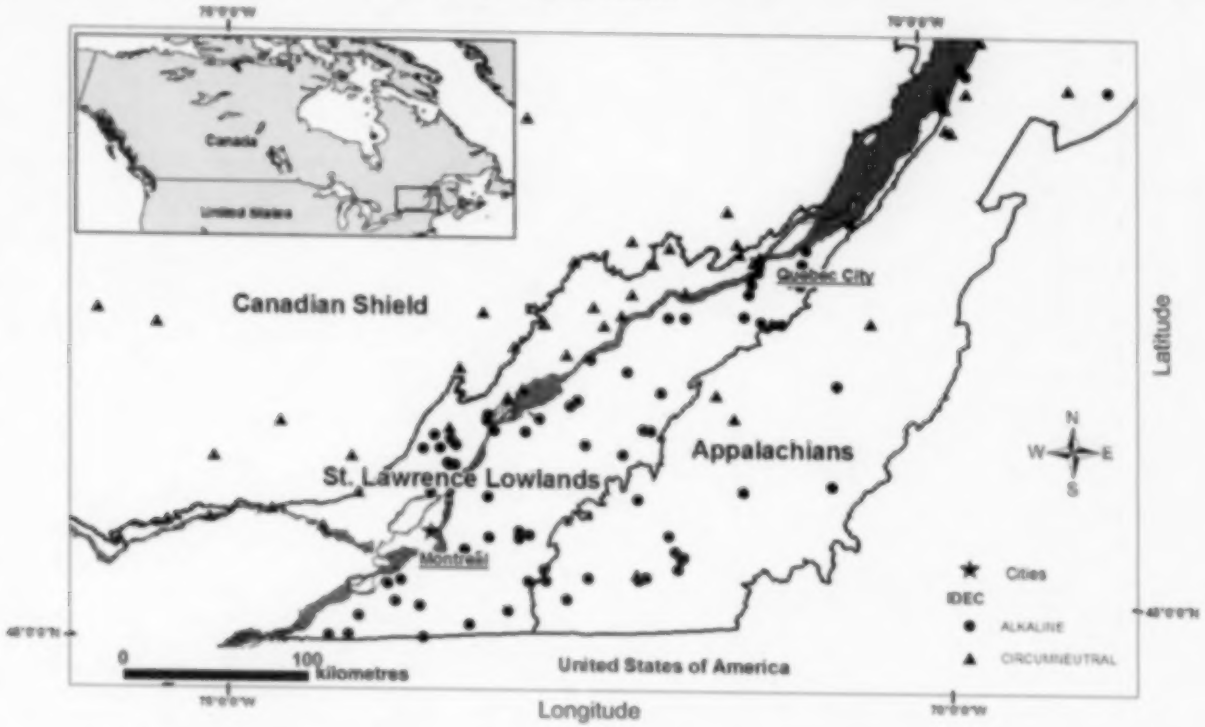
Fig. 1. Ecoregions and sampling locations in the St. Lawrence Basin (Quebec, Canada) for the development of the Eastern Canadian Diatom Index (IDEC). The sites included in the development of the alkaline sub-index are represented by circles; the sites included in the development of the circumneutral sub-index are represented by triangles.



istry through time, seasonal averages were used in the CCAs and the principal component analysis (PCA) to have a better integration of the water chemistry conditions characterizing the diatom sampling period (late summer). Averages were calculated from the six measurements taken in August and September over a 3-year period, including the year the diatoms were sampled. These seasonal averages explain more variance in diatom community structure than one-time chemistry measurements and 2-month averages, because of the variability associated with point measurements (Campeau et al. 2005). Some variables were transformed to improve normality.

Most sites were visited twice for benthic diatom sampling during the fall of 2002 and 2003, resulting in 204 samples. Benthic diatom communities were collected by scraping the top surface of rocks using a toothbrush (see Kelly et al. (1998) for details on routine sampling of diatoms). One composite sample of five rocks (Winter and Duthie 2000) per site was collected within a ~5 m^2 area. Sampling depth varied from 20 to 50 cm, depending on turbidity and water level. The algae were collected from riffles where possible. The algae collected were preserved with Lugol's iodine and stored until the samples were processed. The samples were digested in 30% hydrogen peroxide and mounted on microscope slides using Naphrax. A minimum of 400 valves (Prygiel and Coste 1993) per slide was counted and identified to species level where possible. Taxonomic identifications generally followed Krammer and Lange-Bertalot (1986, 1988, 1991*a*, 1991*b*), Reavie and Smol (1998), Fallu et al. (2000), Krammer (2000, 2002, 2003), and Lange-Bertalot (2001). Diatom community structure is often expressed as species relative abundance. However, because of their low biomass, the importance of abundant small species may be overestimated relative to larger species. In a previous study (Lavoie et al. 2006), diatom metrics expressed as relative abundance, biovolume, and surface area were tested. The results showed that biovolume and cell surface area calculations added substantially to the total analysis time because of the numerous measurements required, but did not improve the variance explained in community structure. This study showed that relative abundance is the most suitable metric to use for developing our diatom-based index.

The influence of ecoregions

A preliminary CCA including environmental variables revealed that the diatom communities sampled were strongly influenced by (*i*) agricultural pollution and (*ii*) pH variations related to the difference in watershed geology between ecoregions (Fig. 2). The strong influence of ecoregions on diatom communities was problematic for the development of the index. Stream reaches in their natural conditions (not influenced by anthropogenic activities) having similar watershed (e.g., geology, surficial deposits, and wetlands) and habitat (e.g., substrate, shade, and current velocity) characteristics should have comparable benthic diatom communities,

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Table 1. Median values and first (Q1) and third (Q3) quartiles for measured water chemistry variables for each ecoregion.

		Canadian Shield			St. Lawrence Lowlands			Appalachians		
Code	Description	Q1	Median	Q3	Q1	Median	Q3	Q1	Median	Q3
TP	Total phosphorus ($\mu g \cdot L^{-1}$)	14.5	17.3	19.8	25.8	50.6	131.3	21.6	24.0	35.5
TN	Total nitrogen ($\mu g \cdot L^{-1}$)	181.7	210.0	258.3	330.0	633.3	1353.3	274.6	410.3	560.4
NH_3-N	Ammonia ($\mu g \cdot L^{-1}$)	20.0	20.0	25.0	25.0	36.0	56.7	21.5	25.0	50.4
CHLA	Chlorophyll *a* ($mg \cdot m^{-3}$)	1.9	2.5	2.8	3.4	7.3	13.9	2.8	4.2	7.9
pH	pH	7.1	7.3	7.3	7.8	8.1	8.4	7.8	8.0	8.2
CON	Conductivity ($\mu S \cdot cm^{-1}$)	28.7	37.6	69.9	159.7	273.4	393.1	135.8	163.3	233.4
TEMP	Water temperature (°C)	19.2	20.2	20.9	19.7	21.5	22.5	19.8	21.2	22.2
O_2	Dissolved oxygen ($mg \cdot L^{-1}$)	8.7	9.1	9.7	8.2	9.1	10.0	8.7	9.2	9.9
TUR	Turbidity (NTU)[a]	0.8	1.3	2.0	2.5	5.6	10.7	1.4	2.7	4.1
SS	Suspended solids ($mg \cdot L^{-1}$)	2.0	2.4	3.4	4.2	7.3	16.4	2.3	3.3	5.5
CF	Coliforms (CFU·100 mL^{-1})[b]	22.8	43.8	85.8	122.0	277.0	1024.0	55.7	122.6	214.7
DOC	Dissolved organic carbon ($mg \cdot L^{-1}$)	4.0	4.6	5.2	4.7	6.0	7.6	3.9	5.7	6.7

[a]Nephelometric turbidity units.
[b]CFU, colony-forming units.

representing reference conditions. These reference conditions for each stream reach type are important to consider when developing a diatom-based index to avoid misinterpreting the biological information. To account for ecoregion characteristics and natural pH variations, diatom reference communities were determined using Kohonen self-organizing maps (SOMs) and multivariate analyses (Grenier et al. 2006). The Kohonen SOM is one of the most well known neural networks with unsupervised learning rules; it performs a topology-preserving projection of the data space onto a regular two-dimensional space. The SOM allows the classification of sites based on communities' similarity–dissimilarity and describes each group based on an a posteriori comparison with environmental conditions. First, stream reaches were classified as a function of their natural watersheds and habitat characteristics, which in turn influence diatom communities. Diatom communities were then classified based solely on taxa abundance data, independent of environmental variables. Resulting groups were graphically presented in ordinations to interpret, a posteriori, the environmental gradients associated with diatom groups and identify the diatom communities representing the reference conditions of each of the stream reach groups. A short description of each group, as defined by Grenier et al. (2006), is presented (Table 2) along with their reference site. Finally, a classification based solely on diatom reference communities found pH to be the main discriminating factor, regardless of ecoregion and stream type. Although a specific diatom reference community may be identified for each stream reach group, these communities exhibit strong similarities and only two reference communities were therefore used: one for circumneutral conditions (pH 6–7.5) and the other for alkaline conditions (pH 7.5–9.1). This procedure allows the discrimination, for example, between the reference diatom communities associated with neutral–acidic streams located on the Canadian Shield from communities representing least-impacted conditions found in smaller, alkaline, agricultural streams of the St. Lawrence Lowlands. A detailed explanation of the methodology used to determine the reference diatom communities is presented in Grenier et al. (2006).

The two sub-indices described in the following paragraphs were developed based on these reference communities. The IDEC-circumneutral includes the sites that have reference communities characteristic of slightly acidic or neutral environments. These reference communities are typical of the Canadian Shield streams, as well as the sites located in the St. Lawrence Lowlands, which have an important portion of their upstream watershed in the Canadian Shield. Circumneutral communities are also typical of the Appalachian streams, where most of the watershed is composed of silicious rocks. On the other hand, the IDEC-alkaline includes the sites that have reference communities characteristic of environments where pH values are naturally higher than 7.5 (Grenier et al. 2006). These reference communities are typical of the St. Lawrence Lowlands streams and the Appalachian streams overlying carbonated rocks and (or) clay rocks (Grenier et al. 2006). The distinction between the two sub-indices is fundamental in allowing each stream the potential to reach a high IDEC value following complete restoration of their ecosystem.

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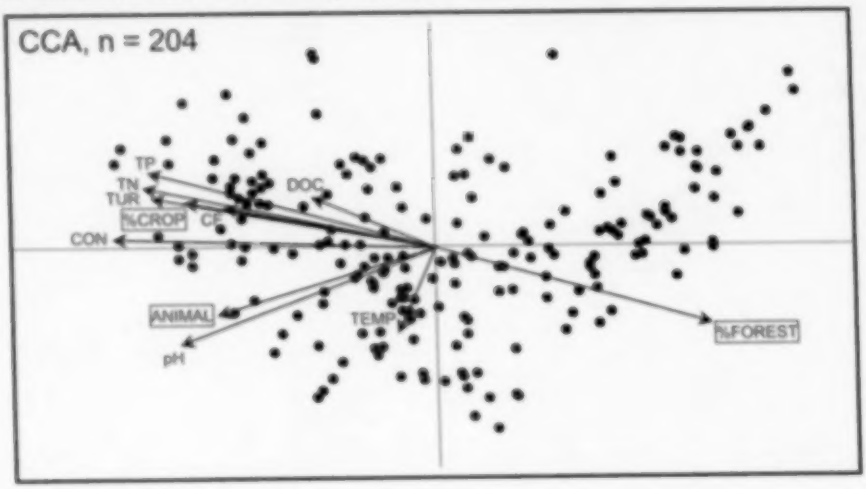
Fig. 2. Canonical correspondence analysis (CCA) site scores for the 204 samples. The land use variables %FOREST, %CROP, and ANIMAL are set as passive variables and show the gradients in forested area (%), cropped area (%), and animal population, respectively. See Table 1 for definitions of other water chemistry variables.



Ordination-based index

Ordinations allow the representation of site (or species) similarity or dissimilarity in a simplified and condensed form, where patterns of variation can be identified and interpreted by extracting the most important dimensions. The method developed in this study is based on CAs where the index value for each site along the gradient of maximum variance is interpreted from its position on the first axis. Although the sites sampled were distributed within three ecoregions, the IDEC was developed based on two sub-indices: IDEC-circumneutral (79 sites) for reference conditions with a pH close to neutral and IDEC-alkaline (125 sites) for the reference conditions characteristic of more alkaline environments. Two CAs were therefore necessary to develop the index. CCA including environmental variables were also conducted for each of the two sub-indices (circumneutral and alkaline) to show primary gradients (see Results). These preliminary CCAs allowed identification of agricultural pollution as the primary gradient influencing species and sites ordination in the CA models (circumneutral and alkaline) used for developing the index. All ordinations were performed using CANOCO 4.5 (ter Braak and Šmilauer 2002). A diatom taxon was included in the analyses if >3 valves were counted within the total set of 204 samples. Diatom data were square root transformed and rare taxa were downweighted. We used Hill's scaling with focus on sample distances (samples scores were weighted means of species scores). Following the CA ordinations, the position of each site along its respective gradient of agricultural pollution (x coordinate) was rescaled to range between 0 and 100. The 5th and 95th percentiles of the x coordinates for the IDEC-alkaline and the 10th and 90th percentiles of the x coordinates for the IDEC-circumneutral were used as the minimum and maximum, respectively, to eliminate extreme values and increase the sensitivity of the index. A high index value represents non- or less-impacted sites, while a low index value represents more heavily impacted sites. The CA-based model can be used to determine the index value for new samples using the formula described in ter Braak and Šmilauer (2002):

$$x_i^* = \lambda^{\alpha-1} \frac{\sum_k w_k^* y_{ik} u_k}{\sum_k w_k^* y_{ik}}$$

where x_i^* is sample position on the first CA axis, λ is first axis eigenvalue, α refers to the scaling method in CANOCO 4.5, w_k^* are species weights, u_k are species scores on the first CA axis, and y_{ik} are species abundances. The CA used to develop the index is based on Hill's scaling with intersample distance, which sets the value for α at 1 (ter Braak and Šmilauer 2002).

Comparing the IDEC with other indices and water chemistry data

The IDEC was compared with the European diatom indices IPS, IBD, TDI, and SLA. As traditional water chemistry measurements may not be appropriate for ecological integrity assessment, comparing the index values with measured environmental variables may not be suitable for testing the ability of the index to inform on the ecological status of a site. Nevertheless, we compared the index value gradient with some measured environmental variables by running a PCA with the index values set as a passive variable. This PCA ordination was performed to evaluate if the direction of the index values follows the environmental gradients. The relationships between IDEC values and nutrient concentrations (TP and TN) were also evaluated. TP and TN concentrations were expressed as seasonal averages.

Generalization capability

To evaluate the accuracy by which the IDEC model can calculate the position (index value) of new samples along the agricultural pollution gradient, a new IDEC model based on

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Table 2. Descriptions of stream reach groups for each ecoregion, including river names, and Eastern Canadian Diatom Index (IDEC) values for each site sampled during the fall of 2002 and 2003.

River	BQMA	IDEC value 2002	IDEC value 2003	Sub-index	Latitude (°N)	Longitude (°W)
Stream reach groups of the Canadian Shield (CS)						
CS 1: sites located at or near lake outlets						
Rivière des Envies (U)*	5030113	72	97	C	46.84	72.54
De la Petite Nation (U)*	4040039	88	100	C	45.90	75.09
Noire (U)*	5040139	100	—	C	46.93	72.12
L'Assomption (U)*	5220017	86	99	C	46.29	73.80
Maskinongé (U)	5260015	56	65	C	46.33	73.36
Du Loup (U)	5280019	61	—	C	46.43	72.97
Du Loup (M)	5280020	58	70	C	46.60	73.19
CS 2: upstream reaches of the St-Charles River						
St-Charles (U)*	5090003	68	94	C	46.86	71.36
St-Charles (M)*	5090016	68	98	C	46.91	71.37
CS 3: upstream reaches of the St-Maurice River and Jacques-Cartier River						
St-Maurice (U)*	5010386	95	—	C	47.56	72.84
Jacques-Cartier (U)*	5080004	100	100	C	47.07	71.42
CS 4: tributaries of the Ottawa River						
Du Nord (U)	4010010	6	—	C	45.91	74.14
Rouge (D)	4020001	74	—	C	45.64	74.69
Du Diable (U)	4020103	54	60	C	46.07	74.63
Du Lièvre (U)*	4060001	95	100	C	46.55	75.50
Du Lièvre (D)*	4060004	57	74	C	45.59	75.42
Gatineau (U)*	4080223	100	100	C	46.62	75.92
Stream reach groups of the Appalachians (AP)						
AP 1: upstream reaches located in the Estrie-Beauce Complex						
Madawaska (U)*	1170001	89	73	A	47.55	68.64
Chaudière (U)*	2340004	100	90	A	46.18	70.72
Chaudière (U)*	2340006	100	82	A	45.69	70.79
Chaudière (M)	2340014	60	57	A	46.50	71.07
St-François (U)	3020035	52	63	A	45.48	71.94
St-François (U)*	3020040	96	99	A	45.37	71.85
St-François (M)	3020081	41	—	A	45.66	72.14
Magog (U)*	3020037	76	62	A	45.27	72.10
Magog (M)*	3020073	80	94	A	45.26	72.16
Magog (D)	3020176	68	77	A	45.40	71.90
Au Saumon (U)*	3020042	96	94	A	45.68	71.40
Massawippi (D)*	3020082	70	77	A	45.36	71.86
Coaticook (D)	3020177	57	50	A	45.31	71.88
Yamaska sud-est (U)*	3030041	100	—	A	45.18	72.66
Yamaska (U)	3030094	73	71	A	45.28	72.51
Yamaska (U)	3030199	19	20	A	45.27	72.80
AP 2: upstream reaches with small watersheds located in the Estrie-Beauce Complex						
Cabano (D)*	1170022	83	66	C	47.58	68.92
Etchemin (U)*	2330010	94	98	C	46.49	70.45
Bécancour (U)	2400005	—	0	C	46.05	71.45
Bécancour (U)	2400006	26	23	C	46.16	71.56
Aux Cerises (D)†	3020187	49	48	C	45.29	72.17
AP 3: upstream reaches located in the Lower St. Lawrence Complex						
Du Loup (U)*	2250002	85	86	C	47.58	69.67
Du Loup (D)	2250005	13	—	C	47.84	69.53
Dufour (U)	2260004	—	0	C	46.55	71.88
St-Denis (U)*	2260005	—	77	C	46.54	71.75
Aux Perles (U)	2260006	—	53	C	47.39	69.87
Des Îles Brûlées (U)	2340086	0	—	C	46.51	71.15

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Table 2 (*continued*).

River	BQMA	IDEC value 2002	IDEC value 2003	Sub-index	Latitude (°N)	Longitude (°W)
AP 4: reaches of the Fouquette Stream						
Fouquette (U)	2E90001	9	11	A	47.71	69.69
Fouquette (D)†	2E90002	32	—	A	47.67	69.66
Stream reach groups of the St. Lawrence Lowlands						
SL 1: reaches of the Richelieu River						
Richelieu (D)	3040009	33	20	A	46.02	73.13
Richelieu (D)	3040010	35	28	A	45.40	73.25
Richelieu (U)†	3040012	49	56	A	45.06	73.33
Richelieu (M)	3040017	35	—	A	45.69	73.19
SL 2: downstream reaches of the St. Lawrence Lowlands with most of the watershed located in the Canadian Shield						
Rivière des Envies (D)	5030114	20	—	C	46.62	72.41
Du Nord (M)	4010002	0	0	C	45.56	74.34
Du Nord (D)	4010008	2	15	C	45.72	74.09
De la Petite Nation (D)†	4040001	26	29	C	45.61	75.13
Gatineau (D)*	4080003	94	83	C	45.49	75.75
St-Maurice (D)*	5010007	77	69	C	46.38	72.61
Shawinigan (D)	5010012	1	0	C	46.54	72.77
St-Maurice (M)*	5010014	78	75	C	46.54	72.75
Batiscan (D)	5030001	43	33	C	46.53	72.34
St-Anne (D)	5040007	29	25	C	46.57	72.21
St-Anne (U)*	5040113	93	100	C	46.90	71.85
St-Anne (M)	5040116	70	66	C	46.82	71.97
Jacques-Cartier (D)*	5080006	66	66	C	46.68	71.75
St-Charles (D)	5090002	38	—	C	46.81	71.26
L'Assomption (D)	5220001	44	43	C	46.04	73.44
Maskinongé (D)	5260003	21	18	C	46.18	73.03
Du Loup (D)	5280001	24	11	C	46.24	72.92
Blanche (D)	5040006	—	0	C	46.03	72.88
SL 3: reaches of the Upper St. Lawrence Lowlands with most of their watershed overlying carbonated rocks						
L'Acadie (D)	3040013	4	13	A	45.43	73.35
Chateauguay (M)	3090003	31	30	A	45.11	74.09
Chateauguay (U)	3090005	35	36	A	45.02	74.17
Trout River (U)*	3090009	100	66	A	45.01	74.30
L'Assomption (M)	5220004	31	33	A	45.94	73.40
De l'Achigan (D)	5220005	0	—	A	45.85	73.45
St-Esprit (D)	5220006	0	10	A	45.86	73.46
Ouareau (D)	5220012	29	27	A	45.95	73.41
Bayonne (D)	5240001	4	7	A	46.09	73.17
SL 4: reaches of small streams with most of their watershed located in the Upper St. Lawrence Lowlands						
Des Hurons (M)	3040007	0	1	A	45.49	73.19
Ruisseau Norton (M)	3090046	27	13	A	45.16	73.68
Des Anglais (U)*	3090047	58	—	A	45.00	73.65
Ruisseau St-Louis (D)	3110003	9	—	A	45.27	73.90
Mascouche (D)	4640003	0	4	A	45.72	73.58
Ruisseau du Point-du-Jour (D)	5220063	4	—	A	45.85	73.41
Ruisseau Vacher (M)	5220239	12	—	A	45.93	73.51
Ruisseau St-Pierre (M)	5220240	0	—	A	45.98	73.44
Ruisseau St-Esprit (M)	5220241	16	—	A	45.93	73.62
SL 5: reaches of small streams with most of their watershed located in the Middle St. Lawrence Lowlands						
Boyer Sud (D)	2300002	26	—	A	46.72	70.98
Boyer Nord (D)	2300003	16	—	A	46.70	71.00
Ruisseau du Portage (D)	2300004	16	—	A	46.79	70.91
Ruisseau Honfleur (D)	2300005	17	—	A	46.69	70.93
Bras d'Henri (U)	2340051	15	27	A	46.54	71.34
Bras d'Henri (D)	2340099	4	—	A	46.51	71.22

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Table 2 (*concluded*).

River	BQMA	IDEC value 2002	IDEC value 2003	Sub-index	Latitude (°N)	Longitude (°W)
Des Pins (D)	3010038	31	37	A	46.00	72.03
Yamaska Sud-Est (D)*	3030031	36	40	A	45.27	72.92
Yamaska Nord (D)	3030108	24	34	A	45.33	72.81
Aux Brochets (D)	3040015	22	41	A	45.12	73.07
La Chaloupe (D)	5230001	6	0	A	46.07	73.18
Aux Perles (D)	2260002	—	2	A	46.29	72.18
Goudron (D)	2260003	—	13	A	46.18	71.95
Gentilly (M)†	—	—	61	A	46.28	72.18
Rosaire (D)	—	—	38	A	46.18	71.95
Du Bois Clair (U)†	—	—	52	A	46.54	71.75
SL 6: reaches of large rivers located in the St. Lawrence Lowlands with most of their watershed in the Appalachians						
Chaudière (D)†	2340033	57	55	A	46.70	71.28
Beaurivage (D)	2340034	36	47	A	46.65	71.30
Bécancour (D)	2400004	58	54	A	46.35	72.44
Nicolet (U)	3010007	52	—	A	46.00	72.09
Nicolet (D)†	3010008	45	50	A	46.15	72.54
Nicolet Sud-Ouest (D)	3010009	15	25	A	46.13	72.60
Nicolet Sud-Ouest (U)	3010036	42	—	A	45.88	72.23
St-François (D)	3020031	51	—	A	46.07	72.82
St-François (D)	3020243	36	39	A	45.93	72.50
Noire (M)	3030003	16	22	A	45.50	72.90
Yamaska (D)	3030023	11	18	A	46.00	72.91
Yamaska (D)	3030026	14	10	A	45.52	72.98
Yamaska (M)	3030123	30	18	A	45.78	72.88
Chateauguay (D)	3090001	22	—	A	45.29	73.80
Des Anglais (D)	3090002	23	27	A	45.18	73.85
L'Assomption (D)	5220003	10	11	A	45.75	73.47
Du Chêne (D)	—	—	34	A	46.55	71.87

Note: The approximate location of the site along the stream is represented by U for upstream, M for middle, and D for downstream. The BQMA (banque de données sur la qualité du milieu aquatique) is the reference number used by the Ministry of the Environment of Quebec. The sub-index used to calculate the IDEC values is represented by the letter C for circumneutral and A for alkaline. See Grenier et al. (2006) for a detailed description of the stream reach groups.

*Rivers represent the reference communities for the group.

†The reference community identified represents the least-disturbed conditions found and is not a true reference community.

a restricted number of samples (59 for the IDEC-circumneutral and 94 for the IDEC-alkaline) was developed. The remaining samples were used to test the new index (20 for the IDEC-circumneutral and 31 for the IDEC-alkaline). The index value of the new sites (test sites), calculated using the IDEC built on a restricted number of samples, were compared with their index value obtained from the IDEC based on the full model (204 samples). This procedure was repeated three times for each sub-index, and the root mean square error (RMSE) was calculated. The comparison between the index values obtained from the full model and those from the test models was used to show that the sites selected for the development of the IDEC covered most of the site diversity found in Quebec and that the index value for new sites can accurately be determined.

Results

Diatom communities

A total of 498 taxa were observed in the streams sampled in Quebec. Most taxa were identified to the species level. Varieties or forms were also noted, and a number was attributed when the identification was not possible. Planktonic taxa were eliminated from the statistical analyses, as well as the taxa represented by fewer than four valves within the total set of 204 samples. Some taxa represented by fewer than four valves were grouped according to their genus. This was the case for certain species of *Caloneis*, *Eunotia*, *Neidium*, *Pinnularia*, and *Stauroneis*. The final diatom matrix included 325 taxa, representing over 98.7% of the total community for each sample.

Diatom communities varied markedly between the least and the most impacted watersheds for both the IDEC-alkaline and the IDEC-circumneutral. The CA ordinations of the species in site space (Figs. 3*b* and 4*b*) show their distribution along the pollution gradient. The high end of the agricultural pollution gradient of the IDEC-alkaline and the IDEC-circumneutral share similar communities dominated by *Cocconeis pediculus*, *Nitzschia palea*, *Navicula gregaria*, *Navicula germainii*, *Amphora pediculus*, *Rhoicosphenia abbreviata*, *Navicula lanceolata*, *Surirella brebissonii*, *Nitzschia inconspicua*, *Navicula tripunctata*, *Fistulifera saprophila*, and *Melosira varians*. These taxa are abundant in the most impacted sites located in the agriculturally im-

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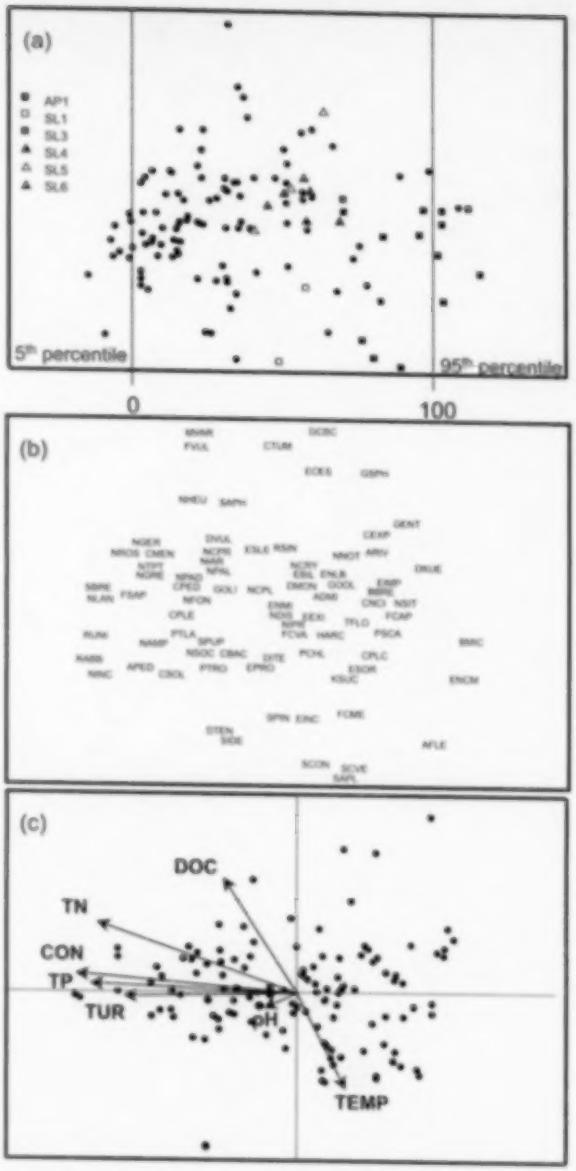

Fig. 3. (*a*) Correspondence analysis (CA) site scores for the 125 samples included in the development of the Eastern Canadian Diatom Index (IDEC)-alkaline. The symbols represent the reference communities for each of the stream reach groups (see Table 2 for group description). The solid circles are nonreference communities. The position of each site along the gradient of agricultural pollution (*x* coordinate) was rescaled to range between 0 and 100. The 5th and 95th percentiles of the *x* coordinates were used as the minimum and maximum to eliminate extreme values and increase the sensitivity of the index. A high index value represents non- or less-impacted sites, while a low index value represents more heavily impacted sites. (*b*) CA species scores for the 125 samples included in the development of the IDEC-alkaline. Only the most abundant taxa are represented in the graph. See Appendix A, Table A1 for a list of the taxa. (*c*) Canonical correspondence analysis (CCA) site scores for the 125 samples included to develop the IDEC-alkaline. The CCA ordination is presented only to show the environmental gradients and was not used to develop the index. See Table 1 for definitions of water chemistry variables.

pacted watersheds of the Lowlands and in the Appalachian streams with a watershed composed of carbonated rocks. These taxa were also found in impacted environments by other authors. For example, *Navicula lanceolata* and *Navicula gregaria* are known to be tolerant to eutrophic conditions (Rott et al. 1998; Kelly 1998) and organic pollution (Kelly 1998). *Rhoicosphenia abbreviata* was found in high orthophosphate concentrations (Rott et al. 1998). *Fistulifera saprophila* and *Nitzschia inconspicua* are characterized by Reavie and Smol (1998) as taxa growing in highly polluted environments and under high concentrations of suspended solids. *Cocconeis pediculus* grows in high phosphorus concentrations and high turbidity (Rott et al. 1998). Many authors have characterized *Nitzschia palea* as a taxon found in hypertrophic conditions (e.g., Rott et al. 1998; Van Dam et al. 1994). *Nitzschia palea* is also N-heterotrophic and tolerant to high turbidity (Leland and Porter 2000) and is an indicator of organic pollution (Kelly 1998). *Ampora pediculus* is a taxon found at high phosphorus levels (Wunsam et al. 2002) but is intolerant to organic pollution (Kelly 1998).

The low end of the agricultural pollution gradient of the IDEC-alkaline and the IDEC-circumneutral also have some taxa in common dominated by *Tabellaria flocculosa*, *Brachysira neoexilis*, *Brachysira microcephala*, and *Encyonema microcephala*. However, the IDEC-circumneutral includes many species of the genus *Eunotia*, while the IDEC-alkaline does not include many *Eunotia* spp. On the other hand, *Nitzschia sinuata* var. *tabellaria* is abundant at the low end of the pollution gradient in the IDEC-alkaline, but is not included in the IDEC-circumneutral. All of these taxa were observed in the sites located on the Canadian Shield or in the less impacted areas of the Appalachians and the Lowlands. The ecology of these taxa is consistent with the information found in the literature. For example, *Tabellaria flocculosa* was found in oligotrophic and slightly acidic environments (Van Dam et al. 1994). *Brachysira brebissonii* grows in oligotraphentic environments, while *Brachysira microcephala* is found in oligo-mesotraphentic conditions (Van Dam et al. 1994). According to the list found in Van Dam et al. (1994), most of the taxa belonging to the genus *Eunotia* are found in oligotraphentic to oligo-mesotraphentic environments. Finally, *Nitzschia sinuata* var. *tabellaria* is a pollution-intolerant taxon (Prygiel and Coste 1993).

Environmental gradients

The CCA ordination for the 204 samples collected during the fall 2002 and 2003 is shown (Fig. 2). The eigenvalue for the first axis (λ_1) is 0.31 and is 0.07 for the second axis (λ_2). These two first axes explain 10.8% of the total variance in diatom communities and 67.7% of the canonical variance (the percentage of the diatom community variance explained by the first two axes). The graph shows that TP, TN, CON, pH, TUR, CF, and DOC are associated with the

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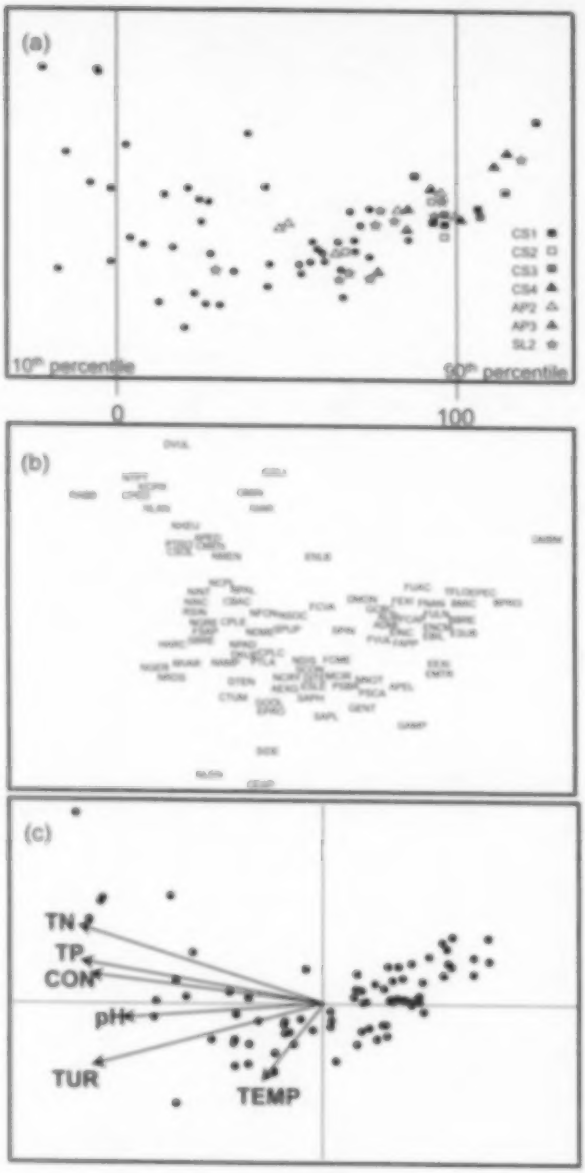

Fig. 4. (*a*) Correspondence analysis (CA) site scores for the 79 samples included in the development of the Eastern Canadian Diatom Index (IDEC)-circumneutral. The symbols represent the reference communities for each of the stream reach groups (see Table 2 for group description). The solid circles are nonreference communities. The position of each site along the gradient of agricultural pollution (*x* coordinate) was rescaled to range between 0 and 100. The 10th and 90th percentiles of the *x* coordinates were used as the minimum and maximum to eliminate extreme values and increase the sensitivity of the index. A high index value represents non- or less-impacted sites, while a low index value represents more heavily impacted sites. (*b*) CA species scores for the 79 samples included in the development of the IDEC-circumneutral. Only the most abundant taxa are represented in the graph. See Appendix A, Table A1 for a list of the taxa. (*c*) Canonical correspondence analysis (CCA) site scores for the 79 samples included to develop the IDEC-circumneutral. The CCA ordination is presented only to show the environmental gradients and was not used to develop the index. See Table 1 for definitions of water chemistry variables.

first axis and that the gradient of maximum variance influencing diatom communities is an agricultural pollution gradient. pH is partly associated with the first and second axes. The second axis is also associated with TEMP. The variables CROP, FOREST (percent area of the watershed), and ANIMAL (number of animal units) are set as passive variables and reflect land use gradients. Because two sub-indices were use to develop the IDEC, two additional CCAs were performed to show primary gradients influencing diatom communities for the alkaline and circumneutral sub-indices (Figs. 3*c* and 4*c*). λ_1 is 0.21 for the IDEC-alkaline and 0.28 for the IDEC-circumneutral. λ_2 is 0.08 for the IDEC-alkaline and 0.08 for the IDEC-circumneutral. For the alkaline sub-index, these two first axes explain 10% of the total variance in diatom communities and 61.8% of the canonical variance. For the circumneutral sub-index, these two first axes explain 11% of the total variance in diatom communities and 62.4% of the canonical variance. These two CCAs show that the first axis (gradient of maximum variance) is associated with agricultural pollution and that the strong natural pH gradient is no longer obscuring the main alteration gradient. Each environmental variable included in the CCAs explained a significant ($p < 0.05$) and independent (variance inflation factor < 10) direction of the variance in diatom data.

The CA-based index

The CA ordinations used to calculate the IDEC-alkaline and the IDEC-circumneutral are shown (Figs. 3*a* and 4*a*). The first axis eigenvalue (λ_1) is 0.29 for the IDEC-alkaline and 0.36 for the IDEC-circumneutral. The second axis eigenvalues (λ_2) is 0.14 for the IDEC-alkaline and 0.13 for the IDEC-circumneutral. These first two axes explain 14.5% of the total community variance for the IDEC-alkaline and 15.1% for the IDEC-circumneutral. A large portion of the total species variance is explained by the environmental variables presented in the CCAs (10% out of 14.5% for the IDEC-alkaline and 11.1% out of 15.1% for the IDEC-circumneutral), and the agricultural pollution gradient (first axis) accounts for the largest portion (7.3% and 8.6%, respectively). As agricultural pollution represents the major gradient influencing diatom communities sampled in this study, the ordinations of samples based solely on diatom data (CAs) shows similar patterns as the CCA ordinations (Figs. 3*a*, 3*c* and 4*a*, 4*c*). It is important to note that the CCA ordinations are presented with the single purpose of showing the major gradients influencing diatom community structures and that the IDEC is strictly determined according to the CA ordinations. The index values for each of the 204 samples were calculated using their coordinate on the first axis of their belonging CA and were rescaled to range between 0 and 100. A list of the streams sampled along with their IDEC value is presented (Table 2). The scale of the index indicates the distance of each diatom communities from

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its specific reference communities. A high index value (80–100) represents non- or less-impacted sites, while a low index value (0–20) represents more heavily impacted sites. The number of sites belonging to five index value classes are shown (Fig. 5) and indicates that most of the sampled communities characterize altered conditions. This result was expected, as most sites included in the Quebec monitoring network are impacted. For the IDEC-alkaline, some of the lowest index values were attributed to small agricultural tributaries and to the Assomption River, Yamaska River, Nicolet River, Bayonne River, and Boyer River. These rivers are well known for their poor water quality and are among the most impacted rivers in the province of Quebec. At the other end of the gradient, the highest values were attributed to Trout River and Yamaska Sud-Est River. These stream reaches are considered among the least impacted in the group of alkaline rivers. For the IDEC-circumneutral, the lowest values were attributed to the Shawinigan River, Du Nord River, Du Loup River, and Des Envies River. These rivers have their headwaters in the Canadian Shield and become impacted as they enter the St. Lawrence Lowlands. The highest values were attributed to the Noire River, Gatineau River, St-Anne River, and Assomption River (upstream). All these nonimpacted rivers originate in the Canadian Shield.

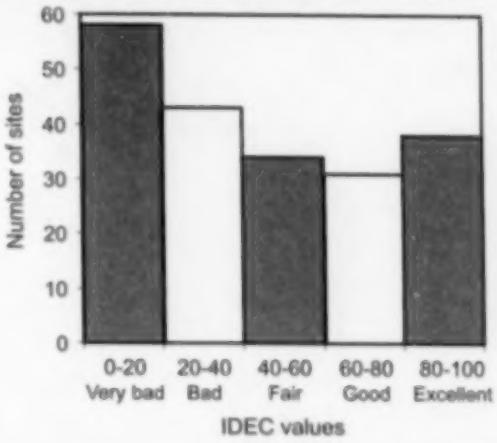
Testing the index

The correlations between the IDEC and the IPS, IBD, TDI, and SLA are shown (Fig. 6). The IDEC is correlated with all the European indices tested, but the environmental gradients sampled in our study seem not to match perfectly with the European environmental gradients used to develop the indices. The most impacted sites were rarely attributed the worst water quality values, indicating that the level of alteration is lower for Canadian streams than for European streams. Moreover, the TDI and SLA indices did not reach values indicating an excellent water quality, showing a limited ability to detect reference conditions in Canada. This result suggests that European environmental gradients are not appropriate for the conditions found in Canada (see Campeau et al. (2005) for a detailed comparison of the IDEC and the European indices).

The gradient in index values was evaluated by its direction on a PCA (Fig. 7), where the samples are ordinated according to the measured physico-chemical variables and the IDEC gradient is set as a passive variable. The PCA eigenvalues were 0.49 for axis 1 and 0.11 for axis 2, indicating that most of the variance is accounted for on the first axis. The first two PCA axes explained 60% of the variance in site distribution. The PCA ordination showed that the gradient of maximum variance (axis 1) is associated with a series of environmental variables reflecting agricultural alteration and that the IDEC varies in the opposite direction. The index values were closely related to the percent cropped land upstream of each site.

The relationship between IDEC values and nutrients (TP: $R^2 = 0.59$, $p \leq 0.05$, $n = 204$; and TN: $R^2 = 0.55$, $p \leq 0.05$, $n = 204$) is shown (Fig. 8). The graphs show that the relationship between IDEC values and nutrients is not linear and that IDEC values vary for the same TP or TN concentrations.

Fig. 5. Number of samples belonging to the five index value classes for the Eastern Canadian Diatom Index (IDEC).



The index's ability to predict the position of new samples on the agricultural pollution gradient was evaluated by developing a new IDEC based on a restricted number of samples. The remaining samples were used to test the predicting ability of the index. This test was repeated three times for each of the two sub-indices. The results showed that the index value for new sites can accurately be predicted from the IDEC. The comparison between the index values obtained from the test models were very similar to the index values obtained using the full model containing 204 samples (RMSE are 1.18, 2.39, and 3.14 for the sub-index alkaline and 1.89, 3.15, and 3.14 for the sub-index circumneutral).

Discussion

Index performance

The effectiveness of the CA in displaying the species' response to an agricultural gradient was clearly shown where the less-impacted sites are distributed at the lower end of the pollution gradient and the most altered sites at the higher end. The two CA ordinations effectively summarized species-specific response to a gradient of agricultural pollution. When placed as passive in a PCA ordination, the IDEC gradient followed the gradient of maximum variance related to agricultural pollution.

The relationship between IDEC values and nutrients (TP and TN) is not linear, indicating that (*i*) there are potentially other stressors than TP and TN influencing the community structure and (*ii*) the index does not only provide information related to the nutrient concentrations, but rather integrates multiple environmental stresses, such as nutrients, ionic content (conductivity), dissolved organic matter, hydrophobic organic compounds, pesticides, etc. This is consistent with the objectives of a biological index that must indicate the integrated response of a community to the overall alteration gradient, such as the one shown in the CCAs and the PCA. The fact that the IDEC values vary for the same concentration in nutrient (TP or TN) may also reflect high algal growth rates that may be depleting ambient nutrient concentrations, resulting in a low nutrient concentration in the water column (Biggs 1990; Dodds 2003).

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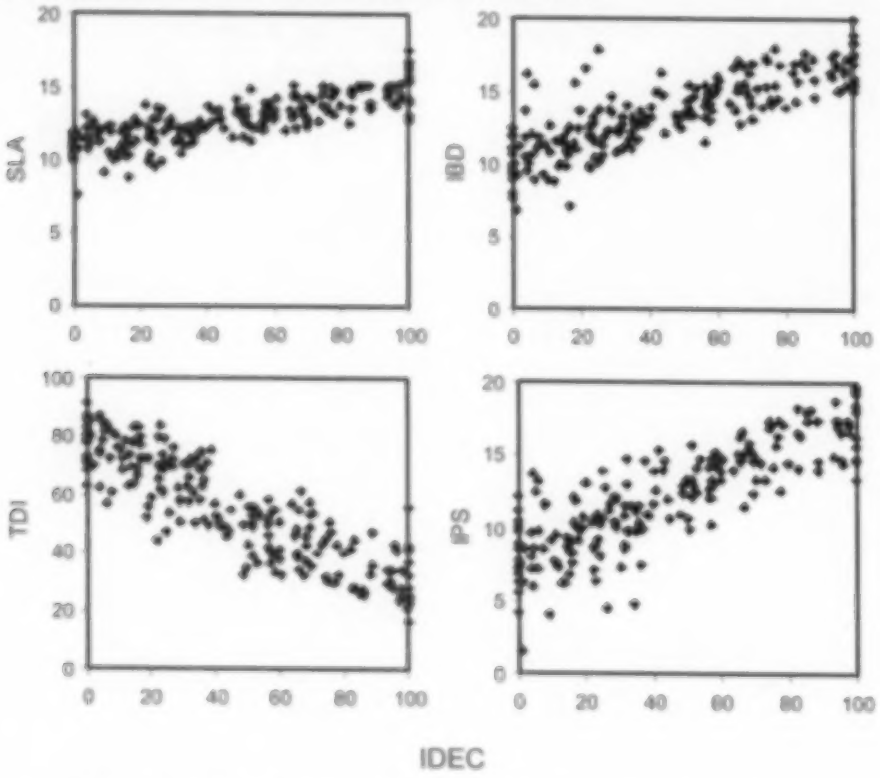
Fig. 6. Correlations between the Eastern Canadian Diatom Index (IDEC) and the Specific Pollus-Sensitivity Index (IPS), the Biological Diatom Index (IBD), the Trophic Diatom Index (TDI), and the Sladecek Index (SLA).



The comparison of the IDEC with selected European indices shows good correlations, indicating comparable information. However, the most impacted sites in Canada were rarely attributed the worst water quality values when using European indices, indicating that the level of alteration may be lower for Canadian streams than for European streams. Also, some European indices do not properly identify Canadian reference conditions, resulting in index values that do not reflect non- or less-impacted communities. We feel that it is more appropriate to develop a new diatom-based index for Canadian stream instead of adjusting and rescaling existing indices to local conditions because (*i*) our index is based on reference conditions and therefore accounts for the influence of ecoregion, (*ii*) the ecological optimum and tolerance of each taxon do not need prior investigation, and (*iii*) the index is independent of water chemistry data.

Dodkins et al. (2005) used a CCA for developing ecological quality assessment metrics for river macrophytes. They identified multivariate species optima along each of the environmental gradients using the CANOCO species-environment file. The tolerances were obtained by repeating the CCA with each variable individually and using the tolerance from the first axis. The optima and tolerances were then used to estimate the value of each environmental variable at the new sites using the weighted averaging formula. This index is closely related to the index proposed in our study for its ordination-based origin. However, compared with Dodkins et al. (2005), our index is entirely based on an ordination and provides the position of sites along the gradient of maximum variance (agricultural pollution) instead of estimated values of environmental variables. As stated by Dodkins et al. (2005), the position of sites along an indirect ordination axis (CA) does not show if a site is impacted, whereas the position along a constrained ordination axis (CCA) shows the direction of the changes in response to an impact. The index proposed in this study was developed to gain information on the position of a site relative to the least- or most-impacted sites. A priori CCAs allowed us to identify the nature and direction of the gradient of maximum variance and permitted us to develop our index based on CAs, where the site ordination along the agricultural gradient is independent of environmental variables. As stated above, species optima and tolerances derived from physico-chemical data imply that biomonitoring is directly linked to environmental variables. The main reason for using biota is that complementary and nonredundant information is provided on ecosystem status. The IDEC was based on CAs to develop a chemistry-free index, where the position of the sites along the gradient of maximum variance (first axis) is strictly determined by diatom community structure and therefore independent of measured environmental variables.

Choosing the gradient analysis method

Choosing the gradient analysis method was not an easy task. We used CANOCO 4.5 (ter Braak and Šmilauer 2002) for its availability and widespread use and considered the of-

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Fig. 7. Principal component analysis (PCA) site scores for the 204 samples. The land use variables %FOREST, %CROP, and ANIMAL are set as passive variables. The gradient in the Eastern Canadian Diatom Index (IDEC) values is also shown as a passive variable. See Table 1 for definitions of water chemistry variables.

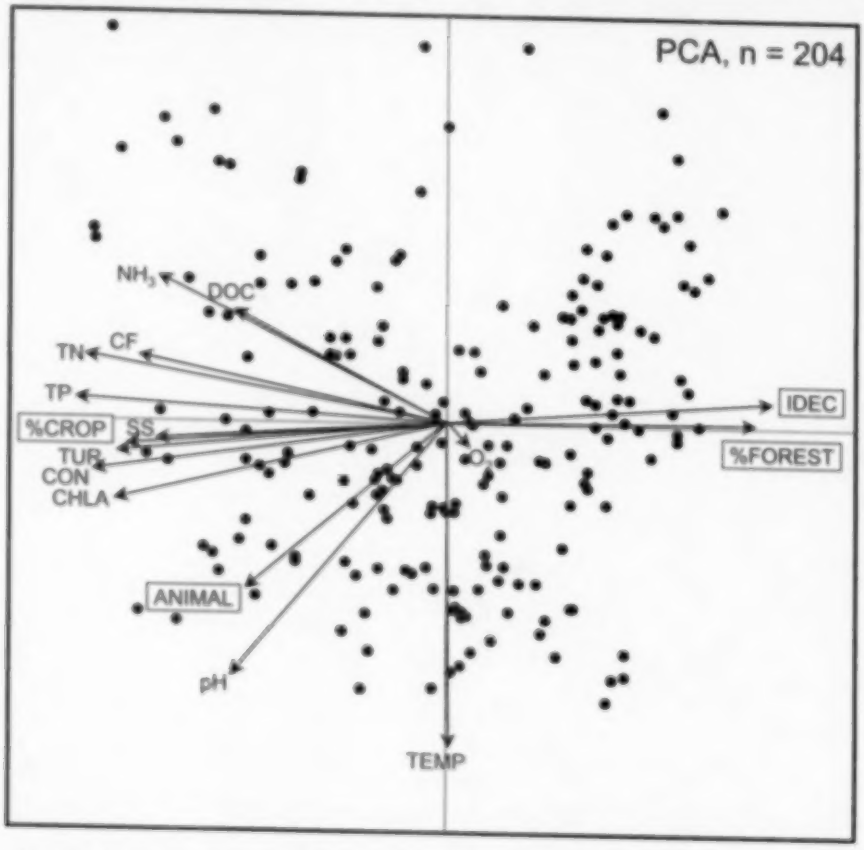
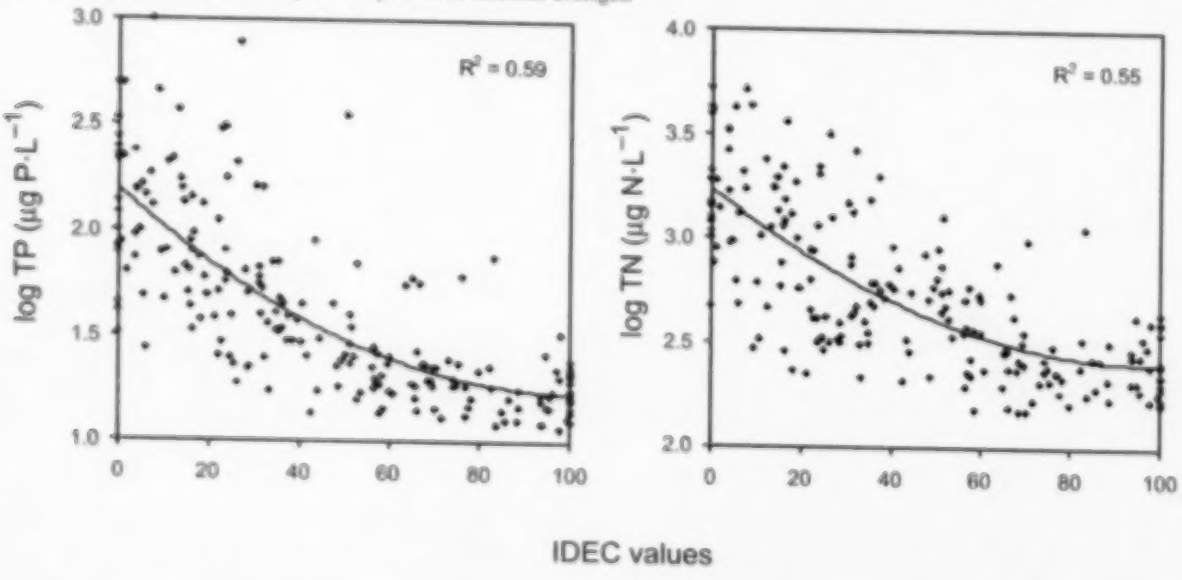


Fig. 8. Relationships between the Eastern Canadian Diatom Index (IDEC) values and nutrient concentrations (TP, total phosphorus; TN, total nitrogen) using chemistry data expressed as seasonal averages.



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fered ordination options CCA, DCA, and CA. Literature findings on the advantages–disadvantages of DCAs oriented our choice towards CA (Minchin 1987; Oksanen 1988; Knox 1989, among others). Well-documented limitations regarding the use of CA include the "arch effect" and the packing of sites at the ends of the gradient (samples at the ends of a gradient may be closer to one another than the sites in the middle of the gradient). Some authors see the curvature as an artifact (e.g., Hill and Gauch 1980; Peet et al. 1988), while others claim that is an important property of successive-replacement data (e.g., Wartenberg et al. 1987; Jackson and Somers 1991). The detrending step in DCA is designed to remove the arch-effect. The first CA axis is not affected by the detrending step. The IDEC was developed using only the first CA axis and is therefore not affected by the detrending procedure. Instability in CA ordinations can arise if the first two eigenvalues have similar values, where their order can be reversed as a result of random variation (Oksanen 1988). The first CA eigenvalues observed in this study are much larger than the second axis eigenvalues, which would unlikely result in a reverse order of axes. The second fault of CA is the compression of samples near the ends of a gradient (because species have their optima outside the studied gradient). DCA includes a rescaling step that equalizes the mean within-sample dispersion of the species score at all points along the gradient (Hill and Gauch 1980). The rescaling of axes has been widely criticized (see Oksanen 1988; Wartenberg et al. 1987; Jackson and Somers 1991) and was therefore not considered as appropriate for the development of the index.

The χ^2 distance is the measure used explicitly in CANOCO 4.5 for CA and DCA analyses. The χ^2 measure incorporates a standardization that emphasizes relative species abundance (Jackson 1993) and is well suited for our species data expressed as relative abundance. However, the χ^2 measure may overemphasize the influence of rare species (Jackson 1993), which means that the difference between the relative abundance for a common species contributes less to the distance than the same difference for a rare species. This limitation was partly overcome by the square root transformation and the down-weighting of rare species. Furthermore, as mentioned in Legendre and Gallagher (2001), the noise associated with the rare species is distributed on the last ordination axes, while our index is only considering the first axis. A diatom index developed with only the most abundant species could help to better reduce the problems related to this limitation.

Future additions

The IDEC presented in this study was developed to provide general information on water quality. As a result, the IDEC does not provide information about the type of alteration that caused degradation at the site. We are currently working on developing a subcomponent to our general index to provide additional information related to the type of alteration present at a site. Moreover, the samples used to develop the IDEC-circumneutral seem to cover the full gradient of environmental conditions, ranging from true reference conditions to very impacted conditions. On the other hand, the IDEC-alkaline is currently lacking true reference conditions for small agricultural streams. Considering that most of the St. Lawrence Lowlands is occupied with farming or urban activities, it was very difficult to identify pristine environments. The sites that are positioned at the lower end of the agricultural pollution gradient represent the least-disturbed conditions. Ongoing surveys to find true reference conditions for the alkaline sub-index will provide new reference sites to include in an upgraded IDEC version. Finally, from a bioassessment perspective, the cheapest, simplest, and fastest approach maximizing the ecological signal is obviously the most attractive for governmental agencies. The IDEC is currently based on 325 taxa, representing over 98.7% of the total diatom community for each sample used to develop the index. Future work will be conducted to evaluate the level to which the IDEC taxonomy can be simplified without significantly altering the index values. A simple, rapid, and easy-to-use diatom-based index would facilitate its integration in Canadian water quality monitoring programs.

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Appendix A

Table A1. List of the codes and names for the most abundant taxa (≥2%).

Code	Taxa	*N*	Min (%)	Mean (%)	Max (%)
MVAR	*Melosira varians* Agardh 1827	84	0.2	8.2	81.5
TFLO	*Tabellaria flocculosa* (Roth) Kützing 1844	84	0.2	4.4	76.7
NCIN	*Navicula* sp. (*N.* cf. *cincta* (Ehrenberg) Ralfs 1861)	50	0.1	4.5	69.9
NITZ8	*Nitzschia* sp. 8	1	68.9	68.9	68.9
NPAL	*Nitzschia palea* (Kützing) W. Smith 1856	166	0.2	2.5	65.4
ADMI	*Achnanthidium minutissimum* (Kützing) Czarnecki 1994	192	0.2	15.3	62.7
NSIT	*Nitzschia sinuata* var. *tabellaria* (Grunow) Grunow 1881	65	0.2	4.8	59.0
NNOT	*Navicula notha* Wallace 1960	80	0.2	3.8	57.2
NAVI5	*Navicula* sp. 5	1	52.4	52.4	52.4
NPAD	*Nitzschia palea* var. *debilis* (Kützing) Grunow 1880	160	0.5	7.8	47.3
CPLE	*Cocconeis placentula* var. *euglypta* (Ehrenberg) Grunow 1884	169	0.2	4.8	44.3
RABB	*Rhoicosphenia abbreviata* (Agardh) Lange-Bertalot 1980	60	0.2	2.4	43.9
NLAN	*Navicula lanceolata* (Agardh) Ehrenberg 1838	74	0.2	2.3	42.2
FCAPF7	*Fragilaria capucina* Desmazières 1825 form 7	69	0.2	2.4	37.2
AMSA	*Achnanthes minutissima* var. *saprophila* Kobayasi & Mayama 1982	150	0.2	3.8	35.8
NINC	*Nitzschia inconspicua* Grunow 1862	94	0.2	3.6	35.8
NFON	*Nitzschia fonticola* Grunow 1879 sensu lato	186	0.2	4.8	33.6
FCME	*Fragilaria capucina* var. *mesolepta* (Rabenhorst) Rabenhorst 1864	48	0.2	3.0	33.1
NERI	*Navicula erifuga* Lange-Bertalot 1985	31	0.2	2.2	32.0
NCPR	*Navicula capitatoradiata* Germain 1981	130	0.1	3.7	30.2
DMON	*Diatoma moniliformis* Kützing 1833	50	0.2	1.4	29.7
FNAN	*Fragilaria nanana* Lange-Bertalot 1991	52	0.2	1.9	29.3
FCAPF6	*Fragilaria capucina* Desmazières 1825 form 6	81	0.2	2.7	24.9
NSSY	*Navicula schroeteri* var. *symmetrica* Patrick 1944	76	0.2	2.9	23.9
EPEC	*Eunotia pectinalis* (Dyllwyn) Rabenhorst 1864	12	0.3	2.6	23.9
CPED	*Cocconeis pediculus* Ehrenberg 1838	94	0.2	1.9	23.1
BMIC	*Brachysira microcephala* (Grunow) Compère 1986	75	0.2	3.0	21.5
GMBM	*Gomphonema manubrium* Fricke 1904	6	0.3	4.8	21.4
ACDF	*Achnanthidium deflexum* (Reimer) Kingston 2003	64	0.2	1.5	20.7
NMINF2	*Navicula minima* Grunow 1880 form 2	68	0.2	2.9	19.9
NAMP	*Nitzschia amphibia* Grunow 1862	99	0.2	2.7	18.8
FCROF2	*Fragilaria crotonensis* Kitton 1869 form 2	1	18.8	18.8	18.8
FCRO	*Fragilaria crotonensis* Kitton 1869	38	0.2	3.1	18.8
CMEN	*Cyclotella meneghiniana* Kützing 1844	117	0.2	3.6	18.7
SPIN	*Staurosirella pinnata* (Ehrenberg) Williams & Round 1987	110	0.2	2.0	18.7
FCAPF5	*Fragilaria capucina* Desmazières 1825 form 5	66	0.1	2.2	18.6
NAVI15	*Navicula* sp. 15	8	0.5	3.0	18.6
ARIV	*Achnanthidium rivulare* Potapova & Ponader 2004	69	0.2	2.7	18.6
SCVE	*Staurosira construens* var. *venter* (Ehrenberg) Hamilton 1992	46	0.2	4.4	18.2
NDES	*Nitzschia* sp. (*N.* cf. *desertorum* Hustedt 1949)	28	0.2	1.9	17.7
ABRY	*Adlafia* sp. (*A.* cf. *bryophila* (Petersen) Moser, Lange-Bert. & Metzeltin 1998)	22	0.2	1.6	17.6
FCAPF8	*Fragilaria capucina* Desmazières 1825 form 8	1	17.3	17.3	17.3
CEXP	*Cymbella excisa* var. *procera* Krammer 2002	22	0.2	2.8	16.8
NCTO	*Navicula cryptotenelloides* Lange-Bertalot 1993	74	0.2	1.5	16.0
NROS	*Navicula rostellata* Kützing 1844	80	0.2	1.9	15.2
NAVI3	*Navicula* sp. 3	40	0.2	1.5	15.1

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Table A1 (*continued*).

Code	Taxa	*N*	Min (%)	Mean (%)	Max (%)
PSBR	*Pseudostaurosira brevistriata* (Grunow) Williams & Round 1987	32	0.2	2.5	14.7
BPRO	*Brachysira procera* Lange-Bertalot & Moser 1994	1	14.5	14.5	14.5
PTRO	*Planothidium rostratum* (Østrup) Round & Bukhtiyarova 1996	8	0.5	4.1	14.2
NVIR	*Navicula viridula* (Kützing) Ehrenberg 1838	52	0.1	0.8	14.2
ENCM	*Encyonopsis* microcephala (Grunow) Krammer 1997	55	0.2	2.3	13.7
NAVI4	*Navicula* sp. 4	22	0.2	1.7	13.7
ESLE	*Encyonema silesiacum* (Bleisch) Mann 1990	129	0.2	1.6	13.4
ACHN1	*Achnanthes* sp. 1	33	0.2	2.2	13.4
NTPT	*Navicula tripunctata* (Müller) Bory 1822	58	0.2	1.1	13.3
APED	*Amphora pediculus* (Kützing) Grunow 1875	79	0.2	1.3	13.1
NGER	*Navicula germainii* Wallace 1960	90	0.2	2.3	12.9
NMINF3	*Navicula minima* Grunow 1880 form 3	37	0.1	1.4	12.3
FCAPF3	*Fragilaria capucina* Desmazières 1825 form 3	80	0.2	2.1	12.3
SBRE	*Surirella brebissonii* Krammer & Lange-Bertalot 1987	73	0.2	1.0	11.9
SCON	*Staurosira construens* Ehrenberg 1843	40	0.2	2.7	10.9
NGRE	*Navicula gregaria* Donkin 1861	136	0.2	2.1	10.4
NDRA	*Nitzschia draveillensis* Coste & Ricard 1980	18	0.2	1.1	10.4
NSOC	*Nitzschia sociabilis* Hustedt 1857	48	0.2	1.1	10.3
NSBM	*Navicula subminuscula* Manguin 1941	60	0.2	1.1	10.2
NRCS	*Navicula recens* (Lange-Bertalot) Lange-Bertalot 1985	41	0.2	1.5	10.0
NILA	*Nitzschia lacuum* Lange-Bertalot 1980	23	0.2	1.8	9.8
FCVA	*Fragilaria capucina* var. *vaucheriae* (Kützing) Lange-Bertalot 1980	148	0.2	1.8	9.7
DVUL	*Diatoma vulgaris* Bory 1824	39	0.2	1.1	9.6
GEIS1	*Geissleria* sp. 1	6	0.3	2.7	9.5
ASHU	*Achnanthes subhudsonis* Hustedt 1921	41	0.1	1.8	9.5
PCHL	*Psammothidium chlidanos* (Hohn & Hellerman) Lange-Bertalot 1999	15	0.2	1.0	9.4
PTLA	*Planothidium lanceolatum* (Brébisson) Round & Bukhtiyarova 1996	126	0.2	1.0	9.3
NCPL	*Nitzschia capitellata* Hustedt 1922	77	0.2	0.9	9.3
BPAR	*Bacillaria paradoxa* Gmelin 1791	15	0.2	1.4	9.3
CNCIF2	*Cymbella* sp. (*C.* cf. *neocistula* (Ehrenberg) Kirchner 1878)	7	0.5	3.1	9.1
FCAPF4	*Fragilaria capucina* Desmazières 1825 form 4	8	0.2	3.2	9.0
NAVI17	*Navicula* sp. 17	1	9.0	9.0	9.0
NELE	*Nitzschia* sp. (*N.* cf. *elegantula* Grunow 1881)	5	0.2	3.9	8.6
NMIN	*Navicula minima* Grunow 1880	167	0.2	1.5	8.5
FULNF2	*Fragilaria ulna* (Nitzsch) Lange-Bertalot 1980 form 2	93	0.2	0.8	8.3
FULNF3	*Fragilaria ulna* (Nitzsch) Lange-Bertalot 1980 form 3	32	0.2	1.6	8.2
NINT	*Nitzschia intermedia* Hantzsch 1880 sensu lato	34	0.2	1.0	8.0
NAVI2	*Navicula* sp. 2	65	0.2	2.3	7.8
TBCO	*Tryblionella constricta* (Kützing) Poulin 1990	36	0.2	1.0	7.8
NIDP	*Navicula insociabilis* var. *dissipatoides* Hustedt 1957	49	0.2	1.0	7.4
NIAR	*Nitzschia archibaldii* Lange-Bertalot 1980	9	0.4	2.3	7.1
NCRY	*Navicula cryptocephala* Kützing 1844	125	0.2	1.2	6.9
FNOP	*Fragilaria neoproducta* Lange-Bertalot 1991	1	6.9	6.9	6.9
NVDA	*Navicula vandamii* Schoeman & Archibald 1987	27	0.1	0.9	6.8
PBND	*Pseudostaurosira binodis* (Ehrenberg) Edlund 2001 sensu lato	6	0.3	2.4	6.8
ESUB	*Eunotia subarcuatoides* Alles, Nörpel & Lange-Bertalot 1991	10	0.2	1.8	6.8
EBIL	*Eunotia bilunaris* (Ehrenberg) Mills 1934	30	0.2	0.8	6.5
NCTE	*Navicula cryptotenella* Lange-Bertalot 1985	81	0.2	0.9	6.5
NZAG	*Nitzschia angustatula* Lange-Bertalot 1987	20	0.2	0.7	6.4
DDEL	*Delicata delicatula* (Kützing) Krammer 2003	25	0.2	1.2	6.4
NDIS	*Nitzschia dissipata* (Kützing) Grunow 1862	110	0.2	1.1	6.3
FAPE	*Fragilaria* sp. (*F.* cf. *perminuta* (Grunow) Lange-Bertalot 2000)	26	0.2	1.7	6.2
NREV	*Nitzschia reversa* W. Smith 1853	4	0.2	2.0	6.1
NITZ6	*Nitzschia* sp. 6	1	6.1	6.1	6.1
GSPH	*Gomphonema sphaerophorum* Ehrenberg 1845	12	0.2	1.0	6.1
FEXI	*Fragilaria exigua* Grunow 1878	5	2.3	3.9	6.0

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Table A1 (*continued*).

Code	Taxa	*N*	Min (%)	Mean (%)	Max (%)
FRAG1	*Fragilaria* sp. 1	16	0.2	1.9	5.9
GENT	*Gomphonema entolejum* Østrup 1903	26	0.2	1.3	5.9
GSCA	*Gyrosigma scalproides* (Rabenhorst) Cleve 1894	25	0.2	0.8	5.9
NIGR	*Nitzschia gracilis* Hantzsch 1860	70	0.2	1.0	5.8
GGRO	*Gomphonema grovei* M. Schmidt 1899	1	5.7	5.7	5.7
CPLC	*Cymophora pulchella* (Ralfs) Williams & Round 1986	18	0.2	1.0	5.7
NLIT	*Nitzschia littoralis* Grunow 1880 sensu lato	21	0.2	1.4	5.6
FCVAF2	*Fragilaria capucina* var. *vaucheria* (Kützing) Lange-Bertalot 1980	21	0.2	1.4	5.5
NAVI23	*Navicula* sp. 23	5	0.2	1.4	5.4
ACHN4	*Achnanthes* sp. 4	3	0.8	2.2	4.9
NITZ4	*Nitzschia* sp. 4	1	4.7	4.7	4.7
AMMO	*Amphora montana* Krasske 1932	21	0.2	0.6	4.7
GOMP1	*Gomphonema* sp. 1	14	0.7	2.0	4.6
NMIC	*Nitzschia microcephala* Grunow 1878	13	0.2	1.3	4.4
ENLB	*Encyonema lange-bertalotii* Krammer 1997 morphotype 1	46	0.2	0.8	4.4
GDEC	*Geissleria decussis* (Østrup) Lange-Bertalot & Metzeltin 1996	63	0.2	0.8	4.3
NSEI	*Navicula seminulum* var. *intermedia* Hustedt 1942	27	0.2	1.2	4.3
TFAS	*Tabularia fasciculata* (Agardh) Williams & Round 1986	7	0.2	1.1	4.3
MCIR	*Meridion circulare* (Greville) Agardh 1831	39	0.2	0.9	4.3
SIDE	*Simonsenia delognei* (Grunow) Lange-Bertalot 1979	21	0.2	0.8	4.3
NMEN	*Navicula* sp. (*N.* cf. *menisculus* Schumann 1867)	72	0.2	0.8	4.2
SSUE	*Surirella suecica* Grunow 1881	8	0.2	1.3	4.2
NIPR	*Nitzschia pura* Hustedt 1954	39	0.2	0.6	4.0
KOJA	*Kobayasiella jaagii* (Meister) Lange-Bertalot 1999	3	0.2	2.3	4.0
CBAC	*Caloneis bacillum* (Grunow) Cleve 1894 sensu lato	46	0.2	0.7	4.0
CTUM	*Cymbella tumida* (Brébisson) Van Heurck 1885	37	0.2	0.6	4.0
DKUE	*Denticula kuetzingii* Grunow 1862	21	0.2	1.1	4.0
HHUN	*Hippodonta hungarica* (Grunow) Lange-Bertalot & al. 1996	15	0.2	1.1	4.0
NLEN	*Navicula lenzii* Hustedt 1936	8	0.2	1.0	3.9
KOSU	*Kobayasiella subtilissima* (Cleve) Lange-Bertalot 1999	3	0.2	1.5	3.9
NTRV	*Navicula trivialis* Lange-Bertalot 1980	50	0.2	0.6	3.9
GCBC	*Gomphonema* sp. (*G.* cf. *cymbelliclinum* Reichardt & Lange-Bertalot 1999)	16	0.2	1.8	3.8
NCLA	*Nitzschia clausii* Hantzsch 1860	19	0.2	0.8	3.7
NACI	*Nitzschia acicularis* (Kützing) W. Smith 1853	50	0.2	0.7	3.5
ABUL	*Amphora* sp. (*A.* cf. *bullatoides* Hohn & Hellerman 1963)	15	0.2	0.9	3.5
DTEN	*Denticula tenuis* Kützing 1844	10	0.2	1.1	3.5
RSIN	*Reimeria sinuata* (Gregory) Kociolek & Stoermer 1987	72	0.2	0.7	3.5
NAGN	*Nitzschia agnita* Hustedt 1957	20	0.2	0.8	3.4
GPUM	*Gomphonema* aff. *pumilum* (Grunow) Reichardt & Lange-Bertalot 1991	50	0.2	0.8	3.4
NCTV	*Navicula* sp. (*N.* cf. *caterva* Hohn & Hellerman 1963)	25	0.2	1.1	3.4
FPYG	*Fallacia pygmaea* (Kützing) Stickle & Mann 1990	8	0.2	1.3	3.4
ADMCF1	*Achnanthidium microcephalum* (Kützing) 1844 form 1	9	0.2	1.3	3.4
SPUP	Complex *Sellaphora pupula* (Kützing) Mereschkowksy 1902	101	0.2	0.8	3.4
NITZ1	*Nitzschia* sp. 1	3	0.2	1.9	3.3
NPRG	*Navicula peregrina* (Ehrenberg) Kützing 1844	37	0.2	0.8	3.3
NAVI20	*Navicula* sp. 20	17	0.2	0.9	3.3
ECAE	*Encyonema caespitosum* Kützing 1849	36	0.2	0.6	3.2
NHEU	*Nitzschia heufleriana* Grunow 1862	24	0.2	0.6	3.2
SLEP	*Staurosirella leptostauron* (Ehrenberg) Williams & Round 1987	7	0.2	0.8	3.2
PTDE	*Planothidium delicatulum* (Kützing) Round & Bukhtiyarova 1996	5	0.5	1.3	3.2
PSAT	*Psammothidium subatomoides* (Hustedt) Bukhtiyarova & Round 1996	33	0.2	0.9	3.1
KLAT	*Karayevia laterostrata* (Hustedt) Kingston 2000	3	0.5	1.9	3.1
NING	*Navicula ingenua* Hustedt 1957	3	0.3	1.3	3.1
FAPP	*Frustulia amphipleuroides* (Grunow) Cleve-Euler 1934	26	0.2	0.6	3.0
GOLI	*Gomphonema olivaceum* (Hornemann) Brébisson 1838	36	0.2	0.8	2.9
FULNF5	*Fragilaria ulna* (Nitzsch) Lange-Bertalot 1980 form 5	10	0.2	0.7	2.9

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Table A1 (*concluded*).

Code	Taxa	*N*	Min (%)	Mean (%)	Max (%)
HCAP	*Hippodonta capitata* (Ehrenberg) Lange-Bertalot & al. 1996	75	0.2	0.8	2.8
PSCA	*Pinnularia subcapitata* Gregory 1856	23	0.2	0.7	2.7
GMIN	*Gomphonema minutum* (Agardh) f. *minutum* Agardh 1831	27	0.2	0.8	2.7
NVEN	*Navicula veneta* Kützing 1844	34	0.1	0.7	2.7
NSAL	*Navicula salinarum* Grunow 1880	6	0.1	0.7	2.7
NRCH	*Navicula reichardtiana* Lange-Bertalot 1989	74	0.2	0.7	2.6
FMST	*Fragilaria* sp. (*F.* cf. *microstriata* Marciniak 1982)	5	0.3	0.9	2.6
STDE	*Stenopterobia delicatissima* (Lewis) Brébisson 1896 sensu lato	8	0.3	0.8	2.5
NAVI10	*Navicula* sp. 10	2	1.6	2.0	2.5
NAVI16	*Navicula* sp. 16	4	1.2	1.8	2.5
DITE	*Diatoma tenuis* Agardh 1812	25	0.2	0.5	2.4
NPHY	*Navicula* sp. (*N.* cf. *phyllepta* Kützing 1844)	27	0.2	0.6	2.4
SSTM	*Sellaphora stroemii* (Hustedt) Mann	7	0.2	1.1	2.3
FVUL	*Frustulia vulgaris* (Thwaites) De Toni 1891	11	0.2	0.6	2.3
EIMP	*Eunotia* sp. (*E.* cf. *implicata* Nörpel, Lange-Bertalot & Alles 1991)	15	0.2	0.6	2.3
EPRO	*Encyonema prostratum* (Berkeley) Kützing 1844	23	0.2	0.7	2.3
AMVC	*Amphora veneta* var. *capitata* Haworth 1974	1	2.3	2.3	2.3
NAVI14	*Navicula* sp. 14	2	0.5	1.4	2.2
GOMP7	*Gomphonema* sp. 7	1	2.2	2.2	2.2
CEXF	*Cymbella* sp. (*C.* cf. *excisiformis* Krammer 2002)	10	0.2	0.9	2.2
SUMI	*Surirella minuta* Brébisson 1849	18	0.2	0.6	2.1
NDIF	*Navicula difficillima* Hustedt 1950	8	0.6	1.5	2.1
FSAP	*Fistulifera saprophila* (Lange-Bertalot & Bonik) Lange-Bertalot 1997	50	0.2	0.6	2.1
NFIL	*Nitzschia filiformis* (W. Smith) Van Heurck 1896	8	0.3	0.9	2.1
BBRE	*Brachysira brebissonii* Ross 1986	24	0.2	0.5	2.0
SDEN	*Stenopterobia densestriata* (Hustedt) Krammer 1987	6	0.3	0.9	2.0
PGRN	*Planothidium granum* (Hohn & Hellerman) Lange-Bertalot 1999	4	0.3	1.0	2.0
NIPM	*Nitzschia perminuta* (Grunow) Peragallo 1903	32	0.1	0.6	2.0
NRHY	*Navicula rhynchocephala* Kützing 1844	33	0.2	0.6	2.0
NAVI22	*Navicula* sp. 22	17	0.2	0.9	2.0
LGOE	*Luticola goeppertiana* (Bleisch) Mann 1990	17	0.2	0.5	2.0
SANG	*Surirella angusta* Kützing 1844	62	0.2	0.5	2.0

Note: *N*, the number of sites where each taxon was observed; Min, Mean, and Max: the minimum, mean, and maximum percentages of each taxon, respectively.

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Implications of long-term dynamics of fish and zooplankton communities for among-lake comparisons

Julian D. Olden, Olaf P. Jensen, and M. Jake Vander Zanden

Abstract: Understanding the environmental determinants of lake community composition has been a central pursuit in freshwater ecology. Previous studies have defined community composition based on temporally limited surveys, with the implicit assumption that interannual variation is negligible compared with among-lake variability. Using a long-term data set for fish and zooplankton communities in five north temperate lakes (Wisconsin, USA), we found that interannual, within-lake similarity in species composition (a measure of temporal stability in community composition) generally exceeded community similarity among lakes. Despite these differences, however, the strength of community–environment relationships were found to range widely (2%–99% explained variation) depending on the choice of single-year sample used in the analysis, a result of high temporal coherence in limnological and biological characteristics. Perhaps of greatest concern, interannual similarity in species composition showed consistent relationships with habitat variables commonly used to explain community differences among lakes. Decreasing lake area and shoreline perimeter (indicative of lower habitat heterogeneity) and seepage lakes were associated with low interannual similarity in community composition, thus confounding the ability to differentiate among lake communities according to their habitat characteristics. In light of our results, we offer a number of explicit recommendations for the selection and analysis of community data in future cross-lake studies.

Résumé : La compréhension des facteurs déterminants de la composition des communautés lacustres représente un objectif de recherche majeur de l'écologie des eaux douces. Des études antérieures ont défini la composition des communautés d'après des inventaires restreints dans le temps, en présupposant de façon implicite que la variation d'une année à l'autre est négligeable par rapport à la variation d'un lac à un autre. À l'aide d'une longue série chronologique de données sur les communautés de poissons et de zooplancton de cinq lacs de la région tempérée nord (Wisconsin, É.-U.), nous trouvons que la similarité d'une année à l'autre de la composition spécifique dans un même lac (une mesure de la stabilité dans le temps de la composition de la communauté) est généralement plus grande que la similarité entre les lacs. Malgré ces différences, cependant, la force des relations communauté-environnement varie considérablement (explication de 2 % à 99 % de la variation) selon le choix de l'échantillon d'une seule année retenu pour l'analyse; c'est le résultat d'une forte cohérence dans le temps entre les variables limnologiques et biologiques. Ce qui est plus inquiétant peut-être est que la similarité de la composition des communautés d'une année à l'autre est constamment en corrélation avec des variables de l'habitat qui sont couramment utilisées pour expliquer les différences de communauté entre les lacs. Les surfaces de lac et les périmètres de rivages plus faibles (des indicateurs d'une hétérogénéité réduite des habitats) et les lacs d'eau d'infiltration sont associés à des similarités faibles de composition de communauté d'une année à l'autre; cela réduit la possibilité de discriminer entre les communautés lacustres d'après les caractéristiques de l'habitat. À la lumière de ces résultats, nous faisons un certain nombre de recommandations explicites pour choisir et analyser les données sur les communautés dans les études futures impliquant plusieurs lacs.

[Traduit par la Rédaction]

Introduction

Lakes are convenient systems for studies in community ecology because they are well bounded and replicated across the landscape (Forbes 1887). The lake, as a result, is considered the de facto observational unit for community analysis, best illustrated by the "lakes-as-islands" analogy for fish (e.g., Barbour and Brown 1974; Browne 1981; Eadie et al. 1986), zooplankton (Arnott et al. 2006), and macrophyte communities (Keddy 1976). Motivated by the growing need

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to understand how freshwater communities respond to environmental change, a substantial body of research in aquatic ecology has focused on establishing relationships between whole-lake habitat attributes and properties of their aquatic fauna (Schindler 2001). These studies have shown that much can be inferred about community composition from lake morphology, trophic conditions, thermal and chemical characteristics, and degree of geographic isolation (e.g., fishes: Minns 1989; Magnuson et al. 1998; Olden and Jackson 2002; and zooplankton: Dodson 1992; Dodson et al. 2000; Rusak et al. 2002). Indeed, many variables have been measured and parameters devised for explaining patterns of fish and zooplankton community composition among lakes at both small and large spatial scales.

Aquatic ecologists and managers have long recognized the difficulties and challenges imposed by the inherent spatial and temporal complexity of lake ecosystems. For example, it is accepted that fish and zooplankton communities show strong within- and among-lake variability in their composition (e.g., Locke and Sprules 1994; Pinel-Alloul 1995; Brind'Amour et al. 2005). Yet surprisingly, previous attempts to quantify associations between species composition and attributes of the environment typically use fish and zooplankton survey data taken in a single year for a given lake, and data from different lakes are often collected across many different years. For example, Olden (2003) used physical and chemical characteristics of 286 north temperate lakes to develop predictive models of fish community composition using single-year fish surveys that were collected over a 20-year period. This study is by no means atypical, and the fish ecology literature is replete with similar examples (e.g., Matuszek and Beggs 1988: 15 years; Minns 1989: 29 years; Mandrak 1995: 92 years). Moreover, studies typically define a lake's fish or zooplankton community based on a single year of sampling, a practice that does not respect the long-term variability in community structure that may characterize many lakes (Magnuson et al. 2006).

Despite this widespread practice in community analyses, little is known of the role that interannual variation in community composition plays relative to the among-lake variation that is typically the focus of these investigations. This raises a number of fundamental questions. How important is within-lake, interannual community variation relative to among-lake variation? If interannual variation is substantial, is the degree of variation related to lake characteristics typically used to explain among-lake differences in species composition? Do these relationships differ among major taxonomic groups, such as fish and zooplankton? Understanding the magnitude of interannual variability in community structure is critical for interpreting the results from studies that relate spatial patterns of environmental variation to community structure, both of which continue to be treated as static entities.

In this paper, we address these questions by examining temporal patterns in fish and zooplankton community composition from five north temperate lakes in northern Wisconsin, USA, surveyed over a 24-year period as part of the North Temperate Lakes Long Term Ecological Research Program (NTLLTERP; see http://lter.limnology.wisc.edu/). Our first objective is to compare within-lake patterns of interannual similarity (a measure of temporal stability in community composition) to patterns of among-lake similarity in fish and zooplankton community structure. Through this examination, we can address the question of whether the communities of neighbouring lakes that vary considerably with respect to habitat availability and complexity, productivity, and degree of landscape connectivity (but share the same regional species pool) are distinguishable despite varying degrees of interannual variation in community similarity. Second, we examine whether the choice of certain combinations of single-year surveys can influence our ability to model community–environment relationships. This addresses the concern of whether single-year estimates of community composition are appropriate for cross-lake comparisons that use data collected from different years. Third, we relate patterns of interannual similarity in community structure to habitat descriptors that are typically used in community–environment studies to describe among-lake differences in species composition. This question explores how the predictive performance of community–environment models may be affected by interannual changes in species composition that characterize many lakes. By reconciling the knowledge gained by addressing these three objectives with known sampling effort and extent needed to characterize fish and zooplankton in lakes (Jackson and Harvey 1997; Arnott et al. 1998), we aim to improve our understanding of the suitability of using the single-year lake survey as the de facto observational unit for community analysis.

Materials and methods

Study sites

Our study uses fish and zooplankton community data for a group of north temperate lakes surveyed extensively over a 24-year period (1981–2004) as part of the Long-Term Ecological Research Program (Magnuson et al. 2006). Data are collected from nine primary lakes and two bogs in Wisconsin, and here we use five lakes located in the Northern Highland Lake District (NHLD), Vilas County, Wisconsin (46°01′N, 89°41′W): Allequash Lake, Big Muskellunge Lake, Crystal Lake, Sparkling Lake, and Trout Lake (Fig. 1; Table 1). We restricted our analysis to these neighbouring lakes to avoid the confounding patterns of spatial and temporal variability caused by gross differences in landscape position, regional species pools, and climate drivers (Rusak et al. 2002). NHLD is within the mixed forest ecoregion of northern Wisconsin, USA, to the south and east of Lake Superior (Martin 1965). The climate of the NHLD is heavily influenced by its proximity to Lake Superior, leading to cool summers and cold winters, and precipitation occurs year-round. The ice-free season typically extends from late April until November, and the frost-free season is from early June until mid-September.

The consistency and intensity of sampling protocols provides the opportunity to investigate interannual differences in fish and zooplankton community structure without the confounding effects of sampling effort and different methods (see Weaver et al. 1993; Jackson and Harvey 1997; although we recognize that field personnel have changed over time). The NHLD has been the recipient of species invasions over the past century, including rusty crayfish (*Orconectes rusticus*) that may influence fish population dynamics but is unlikely to result in the lake-wide extirpation of fish species, and

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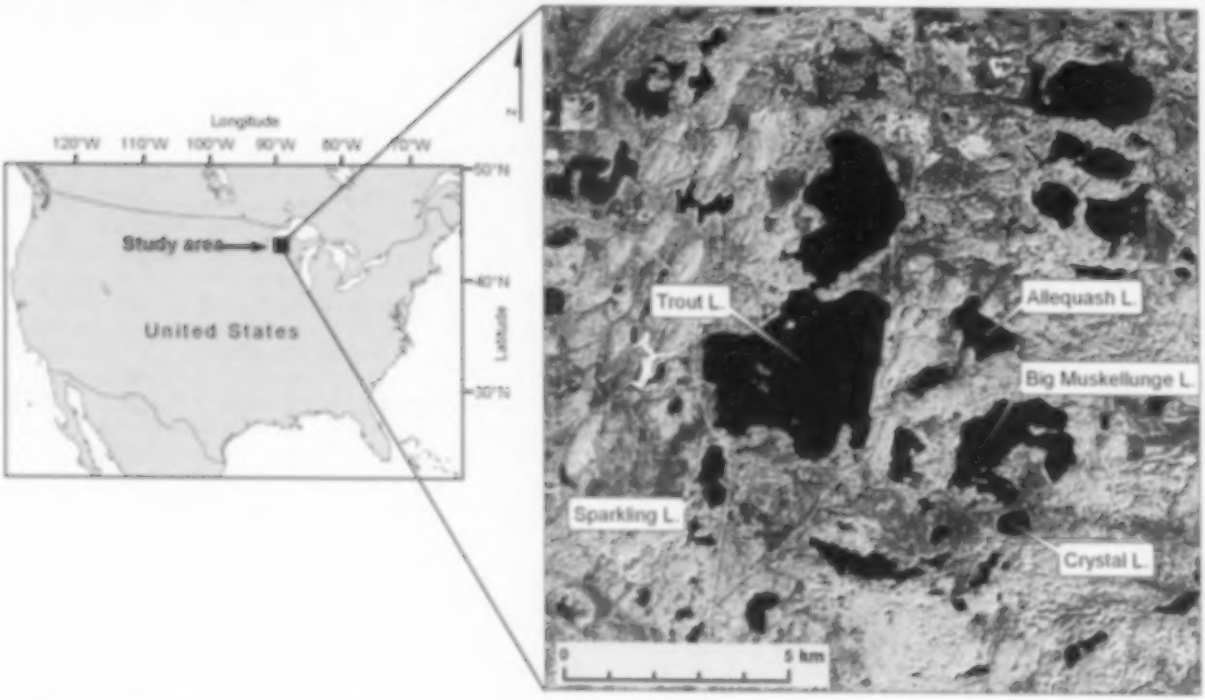
Fig. 1. Location of the five study lakes in the Northern Highland State Forest Region, Wisconsin, USA. Photo courtesy of The Environmental Remote Sensing Center, The Nelson Institute, University of Wisconsin – Madison, Madison, Wisconsin.



rainbow smelt (*Osmerus mordax*) that is thought to have contributed to the extirpation of cisco (*Coregonus artedi*) from Sparkling Lake in 1990 (Hrabik et al. 1998). Our analysis was based on a species-by-site matrix containing records of species' presence or absence for a total pool of 51 fish species and 85 zooplankton taxa (NTLLTERP 2004*a*) collected over a 24-year period in the five study lakes. The lake is commonly used as an operational unit in community analysis, and in this study we refer to a fish or zooplankton community as those species recorded present in a single lake in a single year.

Fish and zooplankton community data

The study lakes were intensively sampled every year using five types of fishing gear (beach seines, trammel nets, fyke nets, gill nets, and boat electroshocking) to determine fish species occurrence. Field protocols are standardized and are described elsewhere (NTLLTERP 2004*b*). The same sampling sites were visited each year, with all surveys conducted between late July and early September. Sampling locations were selected at random in the first year and have remained fixed since then. Three beach seine hauls (each measuring 12.2 m long by 1.2 m deep, with 6.4 mm stretched mesh nylon on the wings and 3.2 mm stretched mesh in the bag) were conducted at night at each of six locations per lake. A 30.5 m long by 1.1 m deep trammel net with an inner panel of 51 mm stretched mesh nylon was deployed for 24 h at two sites per lake. Fyke nets constructed of 7 mm stretched mesh nylon measuring 12 m in length with an 8 m long by 1.25 m deep lead were deployed for 24 h at six sites per lake. Two consecutive 24 h vertical gill net sets (each containing seven panels measuring 4 m wide and 33 m long with stretched mesh sizes of 19, 25, 32, 38, 51, 64, and 89 mm, respectively) were conducted at the deepest point in each lake. Lastly, a boom-style boat electroshocker using pulsed 240 volt, 3–5 amp DC current was used to sample 30 min transects in each lake. For all sampling methods, all fish captured were identified to species and counted. Fish community composition was defined by presence or absence of individual species from the entire data set (i.e., all gears combined).

Zooplankton samples were collected biweekly from June to September at the deepest part of each lake to determine zooplankton presence or absence. Field protocols are standardized and are described in greater detail elsewhere (NTLLTERP 2004*c*). A 45 L Schindler–Patalas trap with 53 µm mesh was used to collect samples at 2 m depth intervals from the surface to the bottom. Samples were preserved in ethanol, hypsometrically pooled (i.e., a subsample was taken from each depth interval in proportion to the volume represented by that depth interval), and two replicate 1 mL subsamples were taken. For this study, we pooled data from the two subsamples. All macrozooplankton were identified to species where possible, and otherwise to genus.

Statistical analyses

We compared fish and zooplankton community composition among lakes across the 24 years by conducting separate analyses of similarity (ANOSIM) and nonmetric multidimensional scaling (NMDS) (PRIMER software, v. 5.2.9,

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Table 1. Characteristics of the five north temperate lakes examined in our study.

Characteristic	Lake				
	Crystal	Sparkling	Allequash	Big Muskellunge	Trout
Location					
Latitude (°N)	46°00′	46°00′	46°02′	46°01′	46°01′
Longitude (°W)	89°37′	89°42′	89°37′	89°37′	89°40′
Morphology					
Area (ha)	36.7	64.0	168.4	396.3	1607.9
Mean depth (m)	10.4	10.9	2.9	7.5	14.6
Maximum depth (m)	20.4	20.0	8.0	21.3	35.7
Shoreline length (km)	2.3	4.3	9.5	16.1	25.9
Landscape position	High	Moderate	Low	Moderate	Low
Connectivity	Seepage	Seepage	Drainage*	Drainage*	Drainage*
Chemistry†					
pH	6.0	7.3	7.5	7.3	7.6
ANC (μequiv.·L^{-1})	16	612	795	366	829
Conductivity (μS)	14	80	88	49	93
Total P (μg·L^{-1})	8.6	15.2	29.3	22.5	16.9
Total N (μg·L^{-1})	207	375	364	489	235
SiO_2 (μg·L^{-1})	20	3582	6486	145	4311
Productivity‡					
Secchi depth (m)	7.3	6.1	3.1	6.7	4.6
Chlorophyll (μg·L^{-1})	1.8	2.2	8.3	3.0	3.3
Fish richness (no. of species)	6–12	9–17	17–24	14–22	19–26
Zooplankton richness (no. of taxa)	16–27	18–29	24–32	16–33	16–38

Note: Reported values are 24-year means for chemistry and the range of fish and zooplankton taxonomic richness over the 24-year study period. ANC, acid-neutralizing capacity.

*These lakes are located in the same watershed.

†pH, ANC, and conductivity were calculated from the average of spring and fall mixis sampling; total P, total N, and SiO_2 were calculated from spring mixis sampling.

‡Secchi disk depth was measured between 1 June and 31 August of each year; chlorophyll (surface) was taken from open water season.

Primer-E Ltd, Plymouth, UK). ANOSIM based on Bray–Curtis similarity coefficient (Bray and Curtis 1957) was used to test the null hypothesis of no difference in fish–zooplankton community composition between lakes across years. ANOSIM is a nonparametric, multivariate procedure broadly analogous to analysis of variance (ANOVA) that has been widely used for testing whether or not groups of sites are statistically different in respect to their relative similarities in community composition (Clarke 1993). ANOSIM tests priori-defined groups (i.e., lakes) against random groups in ordination space by calculating the average of all rank similarities among years within lakes (r_w) and the average of rank similarities among years between lakes (r_b). A test statistic, R, is then calculated as $(\bar{r}_b - \bar{r}_w) / 0.5M$, where $M = [n(n-1)]/2$ and n is the total number of samples (Clarke 1993). The value of R lies between –1 and +1. A value of 1 indicates that all years within lakes are more similar to one another than any years from different lakes; a value of 0 indicates that there is no difference among lakes (i.e., representing the null hypothesis); and a value of –1 indicates that all years within lakes are less similar to one another than any years from different lakes (Clarke and Gorley 2001). We conducted 9999 random permutations to assess the statistical significance of R. ANOSIM is particularly suited for this analysis, as we can explicitly test for differences in fish and zooplankton community composition between lakes and among years and compare an interpretable statistic that measures how separate lakes are, on a scale of –1 (indistinguishable) to 1 (all similarities within lakes across years are less than any similarity between lakes).

NMDS is an ordination method that preserves the rank-ordered distances between sample points in ordination space and for our purposes provides a useful approach for visualizing differences in fish and zooplankton community composition across lakes and over time. NMDS uses an iterative approach that rearranges samples in ordination space to minimize a measure of disagreement (referred to as stress) between the compositional dissimilarities and the distance between the points in the ordination diagram (Kruskal 1964). A distance matrix based on the Bray–Curtis (dis)similarity coefficient was then used to ordinate the sample sites in two dimensions using 10 random starts (a comparison of stress versus dimensionality supported the interpretation of two dimensions). Lake–year combinations with similar community compositions are located closer together in multidimensional space, whereas lake–year combinations with dissimilar communities are positioned farther apart (Kruskal and Wish 1978).

We used regression analysis to assess the relationship between within-lake interannual similarity in fish and zooplankton community composition and habitat descriptors of the lakes that are typically used to explain between-lake differences in communities. These descriptors include surface area, maximum depth, shoreline perimeter, Secchi disk depth, landscape position related to elevation (low, moderate,

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Table 2. Results from the analysis of similarity (ANOSIM) illustrating the results from pairwise lake comparisons of zooplankton (upper-right diagonal) and fish community composition (lower-left diagonal).

	Crystal	Sparkling	Allequash	Big Muskellunge	Trout
Crystal	—	0.54	0.89	0.88	0.72
Sparkling	0.67	—	0.74	0.52	0.50
Allequash	0.92	0.93	—	0.59	0.65
Big Muskellunge	0.88	0.67	0.92	—	0.33
Trout	0.94	0.84	0.96	0.92	—

Note: A value of 1 indicates that all years within one lake are more similar to one another than any years from the other lake; a value of 0 indicates that there is no difference among lakes (i.e., representing the null hypothesis); and a value of −1 indicates that all years within one lake are less similar to one another than any years from the other lake. All values were statistically significant at $P < 0.001$.

or high), and connectivity (seepage lake with no stream connections or drainage lakes with stream connections).

We performed a Monte Carlo experiment to address whether the choice of certain combinations of single-year surveys influences the statistical analysis of among-lake differences in fish and zooplankton communities. For each lake, we randomly selected a single year of community data from s consecutive years of records, where $s = 1$ (2004), 2 (2003–2004), 3 (2002–2004), …, 24 (1981–2004). Using this data, we performed separate regression analyses between fish and zooplankton richness and lake surface area, in addition to conducting canonical correspondence analyses (ter Braak 1986) relating fish and zooplankton community composition to three commonly used descriptors of lake habitat: surface area, maximum depth, and Secchi disk depth. The entire process (i.e., random selection of community data and statistical analyses) was repeated 10 000 times. This analysis mimics the common situation in the literature where data spanning a range of years are used in community analyses. A Mantel test was performed to assess the correlation between two distance matrices representing the interlake dissimilarities according to their fish and zooplankton communities.

Results

We found significant among-lake differences in fish community composition (global $R_{ANOSIM} = 0.75$, $P < 0.001$) and zooplankton community composition (global $R_{ANOSIM} = 0.60$, $P < 0.001$), indicating that within-lake similarity across years exceeded among-lake similarity in community composition. Post hoc ANOSIM comparisons revealed that all lakes were statistically distinguishable from each other with respect to both fish and zooplankton community structure (Table 2). The smallest and largest lakes, Crystal and Trout, were the most distinctive in their fish fauna ($\bar{R}_{ANOSIM} = 0.93$ and 0.92, respectively), followed by Sparkling Lake ($\bar{R}_{ANOSIM} = 0.85$), Allequash Lake ($\bar{R}_{ANOSIM} = 0.85$), and Big Muskellunge Lake ($\bar{R}_{ANOSIM} = 0.78$). In contrast, Allequash Lake and Crystal Lake showed the greatest distinction in their zooplankton fauna ($\bar{R}_{ANOSIM} = 0.76$ and 0.72, respectively), followed by Big Muskellunge Lake ($\bar{R}_{ANOSIM} = 0.58$), Sparkling Lake ($\bar{R}_{ANOSIM} = 0.58$), and Trout Lake ($\bar{R}_{ANOSIM} = 0.55$). For all comparisons, lakes showed greater interannual similarity in their fish community composition (Table 2), and there was little concordance in patterns of among-lake similarity between the two taxonomic groups (Mantel standardized $R = 0.33$, $P = 0.35$).

Results from the NMDS corroborate the compositional differences among lakes, but also show that the magnitude of interannual similarity in both fish and zooplankton community composition can be high and can differ greatly among lakes (Fig. 2). For example, although Crystal Lake was the most distinct from the other lakes, it exhibited the lowest temporal similarity in fish and zooplankton community structure (average Bray–Curtis similarity = 64.9% and 62.9%, respectively). In contrast, the fish community of Big Muskellunge Lake was the least distinct from the other lakes, yet showed among the highest interannual similarity (average Bray–Curtis similarity = 83.5%). Not surprisingly, there was a large range in estimates of fish and zooplankton richness among study years (Table 1). Taken together, the ANOSIM and NMDS results indicated that despite low interannual similarity in community composition, among-lake differences in fish and zooplankton faunas were still larger and distinguish the lakes from each other.

Predictor variables that are commonly used in lake community-environment studies (habitat availability, productivity, and degree of isolation) showed varying associations with mean interannual similarity in fish and zooplankton community composition across the study lakes. Despite our limited statistical power associated with small sample sizes, our analysis revealed a number of interesting trends (Fig. 3). Interannual similarity in fish community structure showed a positive, logarithmic association with both lake surface area ($R^2 = 0.52$, $F_{[1,3]} = 3.29$, $P = 0.17$; Fig. 3*a*) and shoreline perimeter ($R^2 = 0.72$, $F_{[1,3]} = 8.02$, $P = 0.06$; Fig. 3*b*), a negative linear correlation with Secchi disk depth ($R^2 = 0.45$, $F_{[1,3]} = 2.48$, $P = 0.21$; Fig. 3*c*), and no relationship with maximum depth ($R^2 = 0.03$, $F_{[1,3]} = 0.01$, $P = 0.94$). Similar, but statistically weaker, trends were observed for zooplankton communities. Interannual similarity in community structure showed a negative linear association with Secchi disk depth ($R^2 = 0.48$, $F_{[1,3]} = 2.73$, $P = 0.19$; Fig. 3*c*), a weak logarithmic relationship with shoreline perimeter ($R^2 = 0.16$, $F_{[1,3]} = 0.61$, $P = 0.49$; Fig. 3*b*), and no trends with lake surface area ($R^2 = 0.20$, $F_{[1,3]} = 0.07$, $P = 0.83$; Fig. 3*a*) and maximum depth ($R^2 = 0.03$, $F_{[1,3]} = 0.10$, $P = 0.78$). Finally, the magnitude of within-lake similarity in both fish and zooplankton community composition increased along a gradient of increasing lake connectivity (Fig. 3*d*). Isolated lakes with no stream connections (i.e., seepage) and positioned in moderate and high elevations of the landscape (i.e., Crystal Lake and Sparkling Lake) exhibited low interannual similarity in community composition compared with those lakes with

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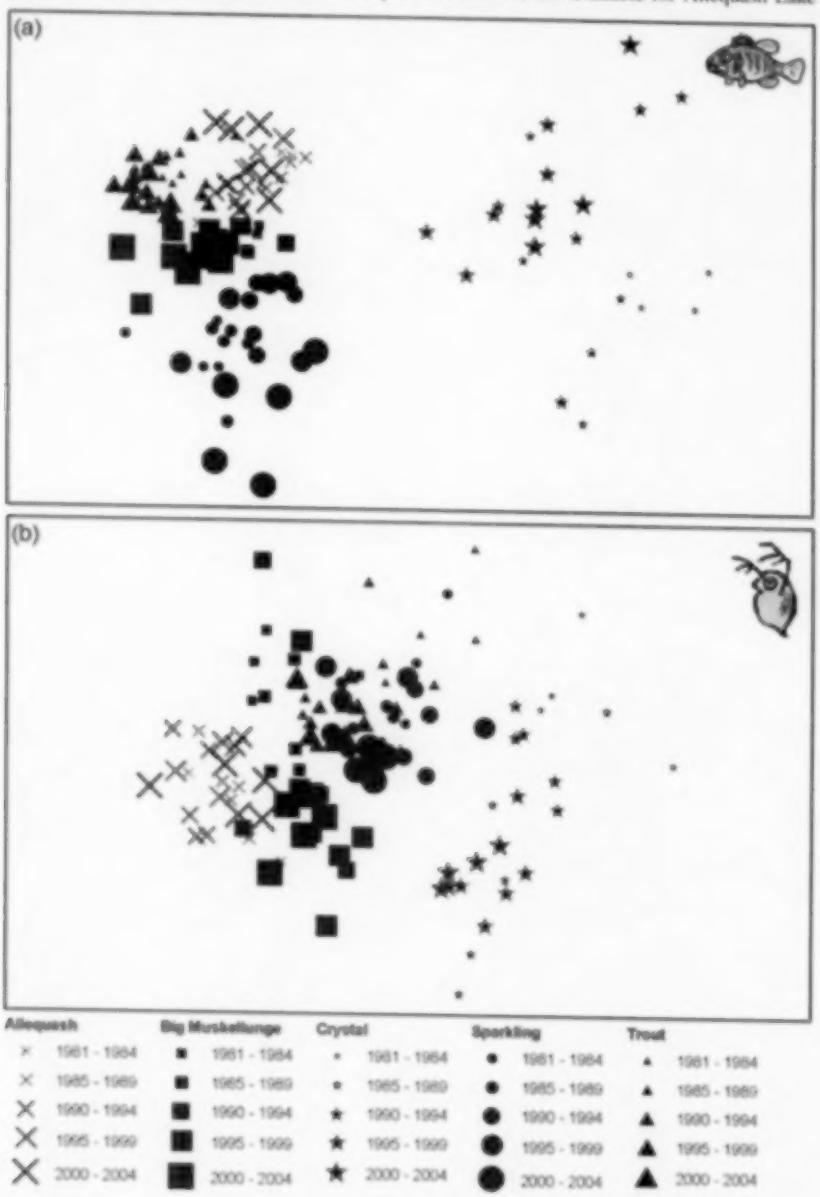
Fig. 2. Spatial and temporal patterns in fish (*a*) and zooplankton (*b*) community composition in the five study lakes sampled from 1981 to 2004, with different symbols for each lake, according to the nonmetric multidimensional scaling based on Bray–Curtis dissimilarity. Stress values were 0.14 for fish and 0.23 for zooplankton. Note that zooplankton data are not available for Allequash Lake in 1981.



stream connections (i.e., drainage) located at lower elevations. However, given the intercorrelations among lake characteristics (e.g., isolated lakes exhibit small surface areas: Table 1), the interpretation of these relationships should be done with care.

Results from the Monte Carlo experiment showed that our ability to explain cross-lake patterns in fish and zooplankton community composition is greatly affected by the choice of survey year. By randomly selecting single-year community data from increasingly longer periods of collection (i.e., 2004, 2003–2004, etc.), we found that the strength of the relationship between lake area and taxonomic richness progressively decreased and became more variable for both fish (Fig. 4*a*) and zooplankton (Fig. 4*b*). We observed similar decreases in the explanatory power of the canonical correlation analysis (CCA) models for fish and zooplankton community structure with increasing periods of collection (fish: 72%–69%; zooplankton: 80%–75%). In summary, a selection of single-year survey data from the entire 24-year period resulted in highly variable area–richness relationships for both fish

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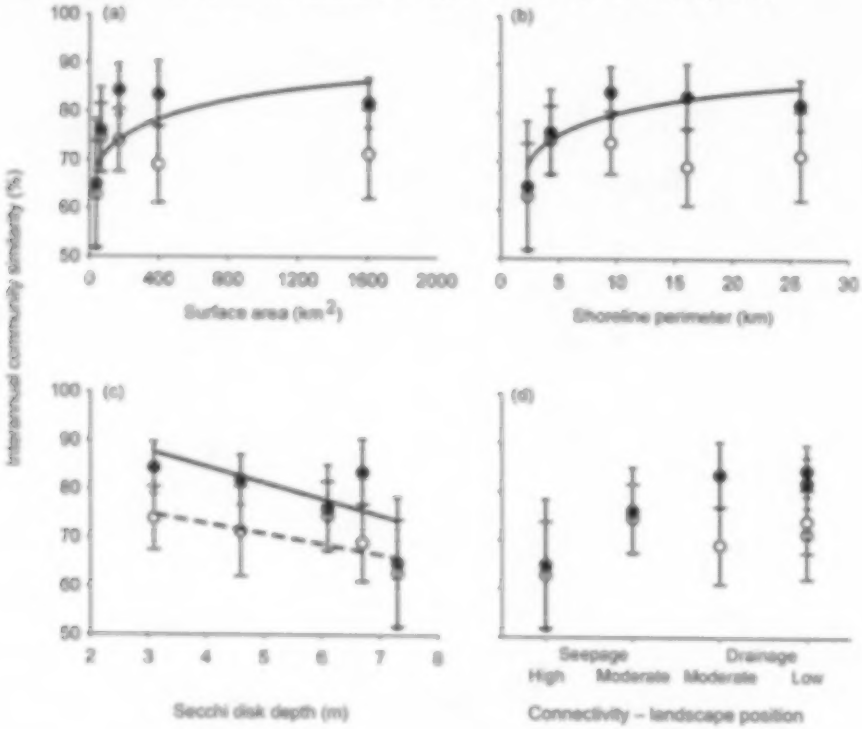
Fig. 3. Mean interannual similarity (Bray–Curtis similarity coefficient) of fish (solid circles) and zooplankton (open circles) community composition versus (*a*) lake surface area, (*b*) shoreline perimeter, (*c*) Secchi disk depth, and (*d*) connectivity–landscape position. Bars represent ±1 standard deviation. Fitted regression lines (fish, solid; zooplankton, broken) are shown for all relationships for which $R^2 > 0.4$. These include for fish ($y = 3.9 \ln(x) + 57.7$, $R^2 = 0.52$) in panel *a*; for fish ($y = 7.1 \ln(x) + 63.3$, $R^2 = 0.72$) in panel *b*; and for both fish ($y = -3.2 \ln(x) + 95.9$, $R^2 = 0.45$) and zooplankton ($y = -1.9 \ln(x) + 81.0$, $R^2 = 0.48$) in panel *c*.



(R^2 = 0.05–0.86) and zooplankton (R^2 = 0.02–0.99), as well as substantial variation in the portion of community variation explained by the CCA models for fish (49.6%–87.5%) and zooplankton (58.3%–89.1%). When repeating this analysis using data collected in the same year (n = 24), we observed decreased explained variability in species richness (fish: R^2 = 0.49–0.85; zooplankton: R^2 = 0.49–0.90) and community composition (fish: 56.0%–80.3%; zooplankton: 68.4%–84.0%).

Discussion

Aquatic ecologists are well aware that lakes exhibit heterogeneity on a broad range of spatial and temporal scales (Hinch 1991; Jackson et al. 2001). In fact, analyses of fish and zooplankton community structure depend on the existence of spatial variability in species composition. While cross-lake studies of community composition have received considerable attention, the extent to which temporal variation in lake community structure, and ultimately the selection of survey data, may influence these modeling endeavours remains unknown. Two common practices in freshwater ecology highlight this concern. First, community composition of a lake is defined according to fish and zooplankton sampling conducted in a single year; second, community–environment analyses are typically based on data sets composed of multiple lakes collectively surveyed over a long time period. Our examination of fish and zooplankton communities in neighbouring north temperate lakes over a 24-year period revealed that change in community similarity among years is both prevalent and differs among lakes. Temporal variation in community composition is likely a result of rare species that may be opportunistic and only periodically exist in certain years; alternatively, these species may be always present but are undetectable using typical sampling techniques during times of low population numbers.

Despite the low interannual similarity within lakes, our results show that the communities of the study lakes were distinguishable from each other. This is an encouraging finding because the lakes examined in this study vary considerably with respect to habitat availability and complexity, productivity, and degree of landscape connectivity (but share the same regional species pool) and therefore are expected to be characterized by different communities. These findings are corroborated by recent studies emphasizing the importance of spatial over temporal variation for differentiating among zooplankton communities in this region (Kratz et al. 1987; Rusak et al. 2002). By extension, our results also suggest that with increasing environmental resemblance of lakes, any differences in community composition among lakes may be dwarfed by high interannual dissimilarity within lakes. Therefore, the suitability of using single-year survey data in

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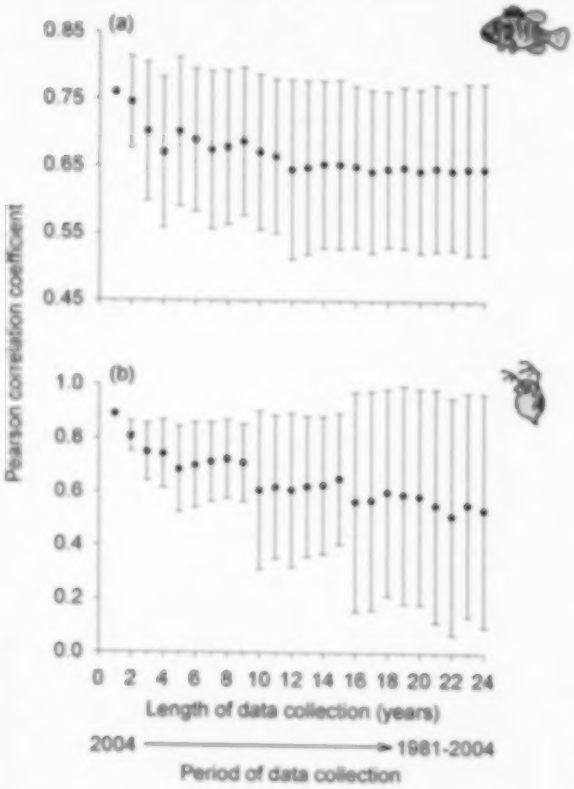
Fig. 4. Pearson's correlation coefficient for the relationship between fish (*a*) and zooplankton (*b*) taxonomic richness and lake surface area according to the random selection of single-year survey data for each lake from increasingly longer lengths of data collection. Circles represent the mean and bars represent ±1 standard deviation from 10 000 Monte Carlo simulations. The single point for 2004 (or 1 year) represents the correlation coefficient using data collected only in 2004, whereas the last point represents the correlation coefficient for using single-year data randomly selected from a 24-year period (1981–2004).



studies of lake community ecology may depend on the degree of between-lake environmental similarity.

Despite overall differences in taxonomic composition among the study lakes, the strength of community–environment relationships for both fish and zooplankton were found to range widely, depending on the length of time over which the lakes included in the analysis were surveyed. Our results suggest that the choice of certain combinations of single-year samples can influence our ability to model community–environment relationships and even affect the familiar species – lake area relationship reported for fish and zooplankton. When community analyses are based on a collection of lakes surveyed over increasingly longer time periods, the strength of the relationship between lake area and richness weakens and becomes more variable. This result is caused by temporal coherence in limnological and biological characteristics among north temperate lakes that are driven by large-scale processes such as climate (Magnuson et al. 1990; Rusak et al. 1999; Willis 2003). As a result, by conducting community analyses with lakes whose communities are characterized using data collected in different years, we are likely to decrease our ability to model between-lake differences in community composition by introducing temporal variability into the analysis (this explains the reduced range of explained variation for analyses conducted with data collected on the same year). This may put into question previous attempts to model community–environment associations using data from lakes surveyed at different points in time. On the other hand, survey data spanning shorter time periods such as collections typically made during a field-based research project may be successfully combined with minimal consequences for community analyses.

Perhaps of greatest concern was our finding that interannual similarity in fish and zooplankton composition showed consistent relationships with habitat variables commonly used to explain community differences among lakes. Decreasing lake area and shoreline perimeter — indicative of lower habitat availability and heterogeneity — was associated with decreasing interannual similarity in community composition. Drainage lakes located at low elevations in the watershed exhibited higher interannual similarity compared with high elevation seepage lakes, suggesting that biotic exchange via stream–lake connections may help temper annual variability in species occurrence resulting from the influence of extrinsic environmental factors (Olden et al. 2001; Havel and Shurin 2004). Though interesting patterns, the sample size of five lakes provides little statistical power, and intercorrelations between environmental variables make it difficult to interpret these relationships. However, focusing on these lakes provides a unique opportunity to address our study objective because of the intensive and consistent sampling of fish and zooplankton communities over 20+ years. Moreover, we are fortunate to have long-term data for lakes located in close geographic proximity to eliminate the confounding effects of temporal differences in community composition caused by regional environmental drivers (Rusak et al. 2002).

The consideration of temporal and spatial variability in aquatic systems is essential for understanding the ecology of freshwater biota (Schindler 2001), but only recently has data become available to address such topics. We offer the following recommendations to ecologists using the lake as an observational unit in community analyses. First, caution should be exercised when using single-year estimates of community structure in cross-lake studies. We found low interannual similarity in fish and zooplankton community composition, which affected our ability to model community–environment relationships. Ecologists who are interested in modeling lake communities without the confounding influence of regional-scale processes such as temporal coherence in climate should pay special attention to the timing of the surveys and focus on analyzing data collected in short time intervals. This approach should enhance our ability to elucidate among-lake differences in community composition driven by lake-specific environmental factors.

Second, because interannual similarity within lakes exceeded among-lake similarity in community composition, single-year estimates of community composition collected over different years may be adequate when studying lakes across a broad environmental gradient. However, as the environmental

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gradient shortens and lakes become more similar in their habitat characteristics, we expect that interannual, within-lake variability will play an increasing role in influencing our ability to model differences among lakes.

Third, our results suggest that the magnitude of interannual community similarity is correlated with environmental variables that ecologists typically use to model between-lake differences in community composition. Future studies can account for temporal variation in community structure by characterizing the species membership of each lake based on data from multiyear surveys. The specific number of samples and years needed to do this are informed by studies such as Arnott et al. (1998), who provided compelling evidence that single-year surveys of zooplankton communities are highly variable and inadequate for characterizing species richness. Similar results were shown for fish communities by Magnuson et al. (1994).

In conclusion, our results generally support the use of single-year surveys in cross-lake studies in freshwater community ecology, but illustrate that understanding the environmental factors driving differences in community structure among lakes is likely to be complicated by among-year differences in species composition within lakes. Future investigations aimed at elucidating the mechanisms underlying these relationships are likely to provide important insight into the resilience of communities to environmental change and ultimately influence how we sample and analyze lake community data.

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Effects of long-distance migration on circulating lipids of sockeye salmon (*Oncorhynchus nerka*)

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Abstract: Lipids circulate as nonesterified fatty acids (NEFAs) or as triacylglycerol (TAG) associated with phospholipids (PLs), the two main components of lipoproteins. Changes in plasma lipid levels of migrating salmon were anticipated in response to the combined demands of exercise and osmoregulation. Circulating NEFAs, TAG, and PLs were measured in sockeye salmon (*Oncorhynchus nerka*) at different stages of their 500 km migration up the Fraser River (British Columbia, Canada). We found that NEFAs represent <7% of total plasma fatty acids (FAs) and show only a minor decrease during migration. In contrast, lipoproteins account for >93% of all the energy of circulating lipids, and concentrations of their main constituents show 27-fold (TAG) and 6-fold (PLs) decreases. The most pronounced change in FA composition of plasma lipids was found in the sample immediately after freshwater transition, with an increase in TAG eicosapentaenoic acid (20:5) and a decrease in TAG palmitic acid (16:0). Lipoproteins are therefore involved in key physiological processes necessary for successful spawning. Previous investigations of lipid metabolism in swimming salmon have focused on plasma NEFAs, but this study shows that shifting attention to lipoproteins should prove more fruitful for future studies of migration energetics.

Résumé : Les lipides circulent comme acides gras non estérifiés (« NEFAs ») ou comme triacylglycérol (TAG) associé aux phospholipides (PLs), les deux composantes principales des lipoprotéines. Chez les saumons migrateurs, la combinaison des besoins en exercice et en osmorégulation laissent prévoir des changements dans les concentrations des lipides plasmatiques. Nous avons dosé les NEFAs, TAG et PLs chez des saumons rouges (*Oncorhynchus nerka*) à différentes étapes de leur migration de 500 km vers l'amont dans le Fraser (Colombie-Britannique, Canada). Les NEFAs représentent <7 % des acides gras plasmatiques totaux et ils ne déclinent que légèrement durant la migration. En revanche, les lipoprotéines constituent >93 % de toute l'énergie des lipides en circulation et leurs composantes accusent des déclins respectivement de 27 fois (TAG) et de 6 fois (PLs). Les changements les plus importants dans la composition en acides gras (« FAs ») des lipides plasmatiques se retrouvent dans l'échantillon prélevé immédiatement après la transition en eau douce, avec une augmentation de l'acide eicosapentaénoïque (20 : 5) de TAG et une diminution de l'acide palmitique (16 : 0) de TAG. Les lipoprotéines sont donc impliquées dans les processus physiologiques essentiels nécessaires pour réussir la fraye. Les études antérieures du métabolisme des lipides chez les saumons en nage se sont concentrées sur les NEFAs du plasma, mais notre étude montre qu'une attention particulière aux lipoprotéines pourrait s'avérer plus productive dans les travaux futurs sur l'énergétique de la migration.

[Traduit par la Rédaction]

Introduction

The "once in a lifetime" spawning migration of sockeye salmon (*Oncorhynchus nerka*) involves swimming for hundreds of kilometres from the open ocean to natal freshwater sites (Foerster 1968). Migration can only be successful if enough energy is stored during the ocean phase because feeding stops upon river entry (Burgner 1991). Several studies have investigated the energy cost of migration (Hinch and Rand 1998; Rand and Hinch 1998; Standen et al. 2002) and have characterized the relative roles of lipids, carbohydrates, and proteins as metabolic fuels for swimming (Idler and Clemens 1959; Gilhousen 1980; Mommsen et al. 1980). In sockeye salmon, lipids and, to a lesser extent, proteins provide most of the energy for migration, locomotory muscles being responsible for the oxidation of 75%–95% of total fat reserves (Gilhousen 1980; Brett 1995). More importantly, most lipids must transit through the circulation before oxidation because most fat stores are located outside of muscle fiber itself, mainly as extramuscular depots, but also as adipocytes

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situated in the connective tissue of the muscle (Kiessling et al. 2004).

Lipids circulate as nonesterified fatty acids (NEFAs) or as triacylglycerol (TAG) associated with phospholipids (PLs), the two main components of plasma lipoproteins. A few studies have investigated the metabolism of plasma NEFAs in wild migrating salmon (sockeye salmon (Ballantyne et al. 1996); Atlantic salmon (*Salmo salar*; Booth et al. 1999)) and captive rainbow trout (*Oncorhynchus mykiss*; Bernard et al. 1999; Weber et al. 2002). However, NEFAs only represent a small fraction of total plasma lipids (Plisetskaya 1980; Babin and Vernier 1989), and prolonged swimming does not increase the turnover rate of NEFAs, at least in rainbow trout (Bernard et al. 1999). Even though lipoproteins are clearly involved in gonad development (Greene and Selivonchick 1987; Wallaert and Babin 1994), they have never been considered as a potential energy shuttle between lipid stores and working muscles. Such a role may have been overlooked because most of the information available on fuel supply to working muscles comes from mammalian studies, and exercising, postabsorptive mammals do not follow this strategy (Terjung and Kaciuba-Uscilko 1986). Instead, they use plasma NEFAs to bring energy to working muscles and maintain very low lipoprotein levels in plasma compared with those in fish (Babin and Vernier 1989).

The consequences of changes in salinity on the plasma lipoproteins of salmon are not well known. The concentrations of TAG and PLs in plasma of smolting salmon are both altered during the freshwater to seawater transition (Sheridan 1988, 1989), but few data are available for adults returning to spawn (Patton et al. 1970; French et al. 1983). The effects of prolonged swimming and salinity changes on plasma lipoproteins have never been investigated in salmon. In this study, our goal was to characterize the plasma lipids containing fatty acids (NEFAs, TAG, and PLs) in wild sockeye salmon and to measure changes in their concentration and composition over a 500 km migration up the Fraser River (British Columbia, Canada). Important changes in lipoprotein metabolism were anticipated in view of their potential roles in shuttling energy to muscle mitochondria and in the response to salinity changes.

Materials and methods

Animal capture and blood sampling

Adult female sockeye salmon (*Oncorhynchus nerka*; 2544 ± 497 g, $n = 35$) from the lower Adams stock (Fraser River, British Columbia, Canada) were caught at four different stages of their spawning migration (Fig. 1). Positive stock identification was achieved by DNA analysis (Beacham et al. 1995, 2004). Ocean-migrating animals were captured with a purse seine in the vicinity of Johnstone Strait (13–19 August 2003). Freshwater-migrating salmon were collected with gill nets at the mouth of the Fraser River (Whonnock, British Columbia; 15 August – 12 September 2003). Prespawning (1 October 2003) and postspawning (15 October 2003) salmon were caught with a beach seine in the Adams River. Immediately after capture, blood samples were collected by caudal puncture. Hematocrit was measured in all animals and found to remain similar at all stages of migration (45%–55%), indicating that observed changes in circulating lipids were not

Fig. 1. Map showing the sampling sites for Adams run sockeye salmon (*Oncorhynchus nerka*) on the Fraser River basin, British Columbia, Canada. Circles indicate the sampling locations: 1, Johnstone Strait (ocean); 2, Whonnock (river entry); and 3, Adams River (pre- and post-spawning).

due to hemodilution. Plasma was separated by centrifugation (800*g* for 10 min) and stored at –80 °C until analysis. The results of this study are based on plasma lipid analyses for 10 animals per stage (except for ocean-migrating fish, where $n = 5$) that were part of a more comprehensive research project aimed at comparing the reproductive success of different stocks of sockeye salmon in the Fraser River (see Patterson et al. 2004).

Extraction of plasma lipids

Plasma lipids were extracted with a 2:1 (*v*/*v*) mixture of chloroform and methanol (Folch et al. 1957) and centrifuged (2000*g* for 10 min). The pellet was discarded and the supernatant was filtered before adding KCl (0.25%) to help eliminating water-soluble compounds. After shaking, the mixture was centrifuged (2000*g* for 10 min) to separate aqueous and organic phases. The aqueous phase was discarded and the organic phase was dried at 75 °C under N_2. Total plasma lipids were immediately resuspended in chloroform before separating them into three classes.

Separation of neutral lipids (NLs), nonesterified fatty acids (NEFAs), and phospholipids (PLs)

Total plasma lipids were loaded on Supelclean solid-phase extraction columns (100 mg LC NH_2; Sigma, St. Louis, Missouri, USA) that were used to separate NLs, NEFAs, and PLs by sequential elution as described previously by Bernard et al. (1999). In preliminary experiments, TAGs were separated from NLs by thin-layer chromatography (TLC). Results showed that the fatty acid compositions of the complete NL fraction and of the TLC-purified TAG fraction were not significantly different because >80% of NLs are TAG.

Gas chromatography analysis

The concentrations of plasma NEFAs, TAG, and PLs, as well as their FA composition, were measured by gas chromatography after methylation (NEFAs) or acid transesteri-

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fication (TAG and PLs) (Chapelle and Zwingelstein 1984; Abdul-Malak et al. 1989). Heptadecanoic acid (17:0) was used as an internal standard because preliminary experiments showed that this acid is absent from the NEFAs, NLs, and PLs of sockeye salmon plasma. The internal standard was added as a free acid to the NEFA fraction before separation from total plasma lipids and before methylation. For the TAG and PL fractions, 17:0 was added after solid-phase column separation and before transmethylation. The fatty acid methyl esters obtained from each fraction were analyzed on a Hewlett-Packard 5890 series II gas chromatograph (Hewlett-Packard, Mississauga, Ontario) equipped with a Supelco 2330 capillary column (30 m × 0.32 mm internal diameter, 0.2 μm film thickness; Agilent, Mississauga, Ontario), using helium as carrier gas and the temperature conditions described in McClelland et al. (1999). Individual fatty acids were identified by determining exact retention times with authentic standards (Sigma-Aldrich, St. Louis, Missouri). Only FAs representing more than 1% of total fatty acids within each plasma lipid fraction are reported. Concentrations of individual fatty acids were calculated by comparing the surface areas of their respective peaks with the surface area of the internal standard peak.

Calculations and statistical analyses

The double bond index (DBI) was used to express the level of fatty acid unsaturation in each class of plasma lipids. It was calculated as follows (expressing percentages as ratios):

$$\text{DBI} = \frac{\text{average number of double bonds}}{\%\ \text{saturated fat}}$$

The average number of double bonds (also called degree of unsaturation) was calculated as

$$\text{Average number of double bonds} = (1 \times \%\ \text{monoenes}) + (2 \times \%\ \text{dienes}) + (3 \times \%\ \text{trienes}) + \ldots + (n \times \%\ \text{FAs with } n \text{ double bonds})$$

Results were evaluated using one-way analysis of variance (ANOVA). When the assumptions of normality or homoscedasticity were not met, a nonparametric ANOVA based on ranks (Dunn's test) was carried out. Percentages were transformed to the arcsine of their square root before analysis, and all values given are means ± standard deviation (SD).

Results and discussion

Salmon migration occurs mainly at sustainable swimming speeds (Hinch and Rand 1998; Hinch et al. 2002), when muscles favor the use of lipids as a dominant source of energy (Moyes and West 1995; Lauff and Wood 1996). Total energy costs and depletion of body lipids are relatively well understood, but little information is available on the mechanisms of lipid mobilization and oxidation. This study shows that long-distance migration has dramatic effects on the plasma lipoprotein constituents of wild sockeye salmon, implying that these circulating lipids are involved in key physiological processes necessary for successful spawning. In sockeye salmon, plasma lipoproteins account for over 93% of the energy in circulating lipids, and the concentrations of their main constituents (TAG and PLs) show 27- and 6-fold decreases, respectively, throughout the complete migration (Fig. 2). In this paper, our goal was to measure the response of wild salmon in their real environment to avoid laboratory artefacts, and this choice prevented controlling conditions, one variable at a time. Salmon migration requires an integrated response involving changes resulting from exercise, salinity transition, fasting, and reproduction, making it difficult to link observed responses to a single cause. This first attempt to examine the metabolism of lipoprotein fatty acids was therefore to identify promising avenues for future experiments in which individual causes could be monitored separately.

Fig. 2. Changes in (*a*) concentration and (*b*) percent contribution to total plasma fatty acids for nonesterified fatty acids (NEFAs; shaded bars), triacylglycerol (TAG; solid bars), and phospholipids (PLs; open bars) during sockeye salmon (*Oncorhynchus nerka*) migration. To allow comparisons between the three lipid classes, concentrations are all given in μmol fatty acids·(mL plasma)$^{-1}$. Absolute PL and TAG concentrations can be obtained by dividing the values presented in (*a*) by 2 (2 fatty acids per PL) and 3 (3 fatty acids per TAG), respectively. Stages: River, river entry; Pre, prespawning; Post, postspawning. Values are means + standard deviation for 10 fish, except for the ocean stage ($n = 5$). Letters indicate statistical comparisons between the four stages within each lipid class. Bars sharing the same letter are not statistically different. Bars lacking letters or with different letters are different from each other ($P < 0.05$).

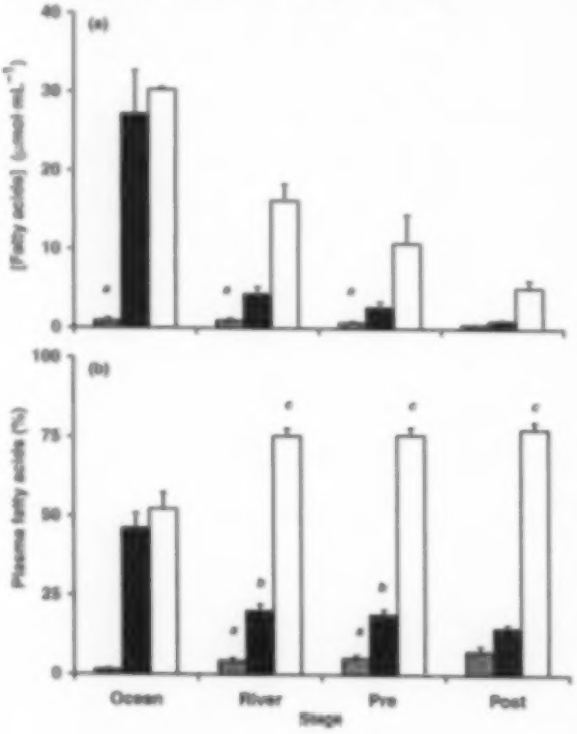

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Table 1. Changes in percentages of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) transported in the plasma of migrating sockeye salmon (*Oncorhynchus nerka*) as nonesterified fatty acids (NEFAs), triacylglycerol (TAG), and phospholipids (PLs).

Fatty acids	Lipid fraction	Ocean	River entry	Prespawning	Postspawning
SFA	NEFA	25.1a (4.0)	34.4a (7.9)	30.6a (6.4)	43.7 (8.6)
	TAG	14.8a (1.9)	8.9 (1.9)	13.1a (0.9)	13.5a (2.6)
	PL	25.0a (0.9)	22.1ab (2.2)	24.0a(1.3)	20.1b (2.1)
MUFA	NEFA	34.2ab (8.7)	25.7b (5.4)	43.0a (5.6)	37.4a (8.5)
	TAG	33.9a (4.2)	35.6a (4.6)	38.8a (6.0)	38.5a (3.8)
	PL	19.9ab (1.2)	18.9a (1.0)	21.5b (1.9)	23.5 (2.0)
PUFA	NEFA	40.7a (5.4)	39.9a (7.3)	26.4 (3.7)	18.9 (2.9)
	TAG	51.3ab (2.4)	55.5a (5.8)	48.2b (6.4)	47.9b (6.1)
	PL	55.1a (1.0)	59.0 (2.3)	54.5a (1.4)	56.4a (1.7)

Note: Values are means for 10 fish (except for the ocean stage where $n = 5$) with standard deviation in parenthesis. Letters indicate statistical comparisons between the four stages within similar FA type and fraction. Values sharing the same letter are not statistically different. Values lacking letters or with different letters are different from each other ($P < 0.05$).

Effects of migration on plasma levels of TAG, PLs, and NEFAs

Changes in the abundance of plasma TAG, PLs, and NEFAs throughout the spawning migration of sockeye salmon are summarized (Fig. 2). Absolute concentrations of the three lipid fractions are shown (Fig. 2*a*), where they are all expressed as fatty acid concentrations to allow comparison. Plasma TAG and plasma PLs showed a strong decrease throughout the spawning migration ($P < 0.001$). TAG decreased by over sixfold from 27 to 4 μmol FA·mL^{-1} between the ocean stage and river entry, before declining more progressively until the end of migration. Plasma PLs decreased from 30 to 5 μmol FA·mL^{-1} throughout the complete migration. Plasma NEFAs stayed constant during most of the migration but showed a small decrease during spawning ($P < 0.05$).

Changes in the percent distribution of total plasma fatty acids between TAG, PLs, and NEFAs are presented (Fig. 2*b*). Together, TAG and PLs accounted for 93%–98% of total plasma FA at all times, whereas the contribution of NEFAs always remained minor (<7% of total plasma FA). The relative importance of TAG decreased from 46% to 15% of total plasma FA during migration, with the most rapid decline occurring between the ocean phase and river entry (46% to 26%). Percent total plasma fatty acid in PLs increased from 52% to 76% during the seawater to freshwater transition and remained at this high level until the end of migration.

Sockeye salmon show a decline in plasma TAG when they move from the ocean to fresh water (Fig. 2). Similar observations have been documented for various salmonids, not only during the spawning migration (Patton et al. 1970; French et al. 1983), but also for smolting animals undergoing the opposite salinity transition (Sheridan 1988, 1989). The timing of the sockeye response suggests that it is related to changes in feeding and (or) environmental salinity. By itself, the cessation of feeding could have this effect because it stops the synthesis of chylomicrons by intestinal cells when the necessary lipid substrates start lacking (Rogie and Skinner 1985; Sheridan et al. 1985; Burgner 1991). However, the effects of fasting and salinity are difficult to separate, even in "controlled" laboratory experiments, because the stress elicited by changes in salinity can strongly affect feeding.

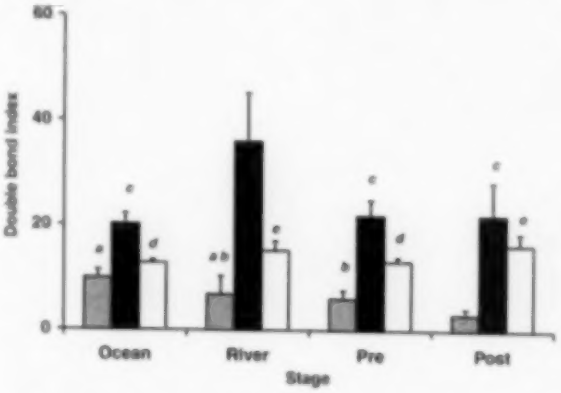

Fig. 3. Double bond index (DBI) for nonesterified fatty acids (shaded bars), triacylglycerol (solid bars), and phospholipids (open bars) in the plasma of migrating sockeye salmon (*Oncorhynchus nerka*). Stages: River, river entry; Pre, prespawning; Post, postspawning. Values are means + standard deviation for 10 fish, except for the ocean stage ($n = 5$). Letters indicate statistical comparisons between the four stages within each lipid class. Bars sharing the same letter are not statistically different. Bars lacking letters or with different letters are different from each other ($P < 0.05$).

Migration also caused a progressive decline in plasma PL concentration (Fig. 2), and this change is consistent with the idea that energy from lipid reserves is transported to working muscles as lipoproteins. Because the declines in PL and TAG levels do not proceed at the same pace, the PL–TAG ratio increases along the migration path, suggesting that the average size of circulating lipoproteins decreases from (large) very low-density lipoproteins (VLDLs) to (smaller and denser) high-density lipoproteins (HDLs). It is interesting to note that the oxidation of VLDLs to support muscle work would progressively convert them to HDLs. In pink salmon (*Oncorhynchus gorbuscha*), it has actually been reported that VLDL levels decrease sharply during migration, whereas HDL levels remain unchanged (Nelson and Shore 1974).

Alternately, it could be argued that the observed declines in TAG and PLs are related to egg production. Vitellogenin

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Table 2. Fatty acid composition (%) in plasma triacylglycerol (TAG) of migrating sockeye salmon (*Oncorhynchus nerka*).

Fatty acids	Ocean	River entry	Prespawning	Postspawning
16:0	9.7a (1.3)	5.3 (2.2)	8.8a (0.6)	8.3a (1.7)
18:0	3.6ab (0.7)	2.6bc (0.6)	2.5c (0.6)	4.1a (0.9)
18:1	24.1ab (3.1)	27.1a (2.7)	25.3ab (2.5)	22.4b (2.7)
20:1	4.1a (0.7)	2.9ab (1.9)	2.0ab (1.1)	1.5b (1.8)
22:1	1.4a (0.6)	2.4a (1.2)	3.2ab (1.4)	12.5b (5.7)
20:5	16.0 (1.2)	21.5a (1.6)	22.8a (3.3)	20.2a (3.2)
22:5	7.0a (0.8)	6.6ab (0.9)	4.9bc (0.8)	3.6c (1.4)
22:6	21.7a (3.0)	17.2b (2.5)	15.6b (2.6)	17.8ab (2.6)
Others*	12.4a (1.5)	14.3a (3.4)	14.8a (8.0)	9.6a (6.0)

Note: Values are means for 10 fish (except for the ocean stage where $n = 5$) with standard deviation in parenthesis. Letters indicate statistical comparisons between the four stages within TAG. Values sharing the same letter are not statistically different. Values lacking letters or with different letters are different from each other ($P < 0.05$).

*"Others" consists of 10–12 fatty acids representing <2% of the total fatty acid content in plasma TAG.

Table 3. Fatty acid composition (%) in plasma phospholipids (PLs) of migrating sockeye salmon (*Oncorhynchus nerka*).

Fatty acids	Ocean	River entry	Prespawning	Postspawning
16:0	17.0ab (0.6)	14.0c (1.7)	17.5a (1.0)	14.2bc (1.9)
18:0	6.6ab (0.6)	6.9a (1.1)	5.7b (0.4)	4.7 (0.6)
18:1	12.7ac (0.9)	12.4c (0.5)	15.5ab (1.4)	16.5b (1.6)
20:1	1.8ab (0.2)	1.3b (1.0)	2.2a (0.5)	2.7a (1.1)
22:1	0.4a (0.1)	1.1b (0.7)	0.5ab (0.1)	0.7ab (0.3)
20:5	12.4ab (0.9)	8.6a (5.6)	15.0bc (1.2)	15.6c (1.7)
22:5	5.5a (0.7)	5.7a (1.1)	6.3a (0.7)	6.0a (0.9)
22:6	33.5 (2.5)	37.3 (2.0)	29.4a (1.6)	29.7a (1.9)
Others*	10.1a (1.0)	12.8ab (6.8)	7.9b (1.6)	9.8a (1.3)

Note: Values are means of 10 fish (except for the ocean stage where $n = 5$) with standard deviation in parenthesis. Letters indicate statistical comparisons between the four stages within PLs. Values sharing the same letter are not statistically different. Values lacking letters or with different letters are different from each other ($P < 0.05$).

*"Others" consists of 8–10 fatty acids representing <2% of the total fatty acid content in plasma PLs.

is the main lipoprotein used to shuttle lipids from storage sites to the gonads (Babin and Vernier 1989). However, it is important to keep in mind that gonad development represents only a minor sink for lipids compared with locomotion. In sockeye salmon, it has been estimated that only 6% of total lipids used during migration is directed to reproduction, whereas the remaining 94% is allocated to locomotion (Idler and Clemens 1959; Brett 1973). Therefore, the large overall response to migration monitored in our study is more likely to reflect the lipid requirements of swimming rather than gonad development.

In fish, changes in plasma NEFAs have frequently been used to evaluate the effects of swimming and reproduction on lipid metabolism (Ballantyne et al. 1993, 1996; Booth et al. 1999). However, NEFAs represent a minor fraction of total FAs in plasma lipids (Fig. 2*b*) and migration only causes a very small decrease in the plasma NEFA concentration of sockeye salmon (Fig. 2*a*). A previous study on rainbow trout demonstrated that endurance swimming does not stimulate NEFA fluxes above resting levels (Bernard et al. 1999). This observation could be explained if the TAG and PL components of lipoproteins, rather than NEFAs, play a prominent role in shuttling energy from storage sites to the muscles of swimming salmonids.

Effects of migration on the fatty acid composition of plasma lipids

After characterizing changes in total plasma TAG, PLs, and NEFAs, the FA composition of these three lipid fractions was examined. Changes in the relative abundance of fatty acids with different degrees of saturation in the three classes of plasma lipids are summarized (Table 1). Overall, the percentages of saturated, monounsaturated, and polyunsaturated FAs (SFA, MUFA, and PUFA, respectively) within each lipid class were relatively constant because migration had only minor effects. However, a few changes were noticeable: (*i*) in plasma NEFAs, PUFA content decreased at the end of migration and SFA content increased after spawning, (*ii*) in plasma TAG, SFA content decreased at river entry, and (*iii*) in plasma PLs, PUFA content increased at river entry. Changes in saturation levels of the three lipid classes throughout migration are summarized (Fig. 3) in the DBI. The most notable changes are a large increase in the DBI of plasma TAG between the ocean phase and river entry (from

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20 to 36) and a progressive decrease in the DBI of plasma NEFAs throughout migration (from 10 to 3). The timing of this response also suggests that TAG from circulating lipoproteins is used to supply FAs for modifying membrane fluidity as the animals experience a rapid change in salinity. Homeoviscous adjustments could therefore be achieved by altering the PUFA–SFA ratio in plasma without changing membrane cholesterol content or the composition of PL head groups (Bell et al. 1986; Hazel and Williams 1990).

The effects of migration on the FA composition of plasma TAG are presented (Table 2). Eight main FAs accounted for >85% of total TAG fatty acids, the remaining being represented by 10–12 minor FAs (1%–2% each). Oleic acid (18:1), eicosapentaenoic acid (EPA; 20:5), and docosahexaenoic acid (DHA; 22:6) were the most abundant FAs in TAG. Overall, the FA composition of plasma TAG remained relatively constant, but some changes were noticed. The decrease in palmitic acid (16:0) and the increase in EPA (20:5) taking place between the ocean phase and river entry were largely responsible for the observed increase in DBI (see Fig. 3). The only PUFA showing a consistent change was docosapentaenoic acid (22:5), the relative contribution of which kept decreasing throughout the migration. The FA composition of plasma PLs is summarized (Table 3). Eight main FAs accounted for >90% of total FAs in plasma PLs, the remaining being represented by 8–10 minor FAs (1%–2% each). DHA (22:6), palmitic acid (16:0), oleic acid (18:1), and EPA (20:5) were the most abundant in plasma PLs. Overall, the FA composition of plasma PLs remained relatively constant, but a significant decrease in the DHA (22:6) was observed between the beginning of migration and the spawning site (Table 3).

Over the migration, we were expecting changes in composition as a result of selective metabolism in target tissues such as muscles, gills, and gonads. Lipoprotein lipase (LPL) has been thoroughly characterized in mammals, where it plays a key role in orchestrating FA mobilization from lipoproteins. LPL shows selectivity for various types of fatty acids and, in particular, is known to release MUFAs preferentially (Goldberg and Merkel 2001). Fish LPL has also been identified (e.g., see Black and Skinner 1987) and generally shares most characteristics with its mammalian counterpart (Lindberg and Olivecrona 2002). It would be surprising if fish LPL did not show selectivity, but this particular property still needs to be investigated. In vitro evidence indicates that fish tissues show selective oxidation of different FAs (Henderson and Sargent 1985; Sidell and Driezdic 1985; Kiessling and Kiessling 1993). For example, MUFAs are the preferred fuel for red muscle mitochondria of Antarctic fish (Sidell et al. 1995), and the amount of oleic acid (18:1) in lipid reserves of Atlantic salmon is correlated with swimming performance (McKenzie et al. 1998). Saturated (16:0) and monounsaturated (18:1) FAs are not incorporated similarly into tissue TAG and PLs because these two fatty acids play different physiological roles (Weber et al. 2002). Taken together, these observations led us to predict that sockeye salmon would show changes in the FA composition of plasma lipids throughout their long-distance migration. Contrary to expectation, very few changes in FA composition were observed in TAG and PLs, and in particular, both lipid fractions kept the same MUFA levels. The exact reasons why FA composition remains relatively constant during long-distance migration are unknown. One possibility is that selectivity affects the flux of MUFAs rather than their concentration, but additional experiments are needed to address this issue (e.g., see Haman et al. 1997).

This study shows that sockeye salmon undergo major changes in plasma lipoprotein metabolism during long-distance migration. The timing and direction of these changes suggest, for the first time, that lipoproteins are used as an important oxidative fuel in fish muscles and possibly play a role in the response to abrupt salinity transitions. Previous investigations of lipid use by migrating salmon have focused on plasma NEFAs as the only shuttle system between fat stores and working muscles. Results from this study shift attention to lipoproteins because (*i*) migration affects them most and (*ii*) they account for over 93% of total plasma lipids. The exact contributions of this pathway to locomotion, osmoregulation, and reproduction remain to be established. In particular, quantifying the effects of prolonged swimming on lipoprotein turnover and LPL activity are promising areas for further research.

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The effects of isolation and colonization history on the genetic structure of marine-relict populations of Atlantic cod (*Gadus morhua*) in the Canadian Arctic

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Abstract: The genetic consequences of extended periods at low population size are fundamental to the conservation of depleted species such as the Atlantic cod (*Gadus morhua*). We compared microsatellite genetic variability among cod populations in Canadian Arctic lakes with that of Gilbert Bay resident and inshore cod from eastern Canada. The Arctic populations had the lowest genetic diversity and were the most strongly genetically structured and distinct. By contrast, eastern Canadian samples expressed high allelic diversity and were not significantly genetically structured or distinct relative to each other, whereas Gilbert Bay resident cod were intermediate to the Arctic and eastern Canadian groups. Our results are consistent with the hypothesis that the Arctic populations were colonized between 8000 and 5000 years ago and have experienced little or no gene flow since that time. Despite isolation at the extreme of the species' range, the Arctic populations have retained relatively high heterozygosities and high genetic effective population sizes relative to census sizes (N_e–N_c ratios). Potential explanations for this include the absence of fishing pressure, allowing for the persistence of large, highly fecund individuals, as well as biotic (e.g., absence of planktivores) and abiotic (e.g., low environmental stochasticity) factors in the Arctic lakes that minimize individual variance in reproductive success.

Résumé : La connaissance des conséquences génétiques de longues périodes de faible densité de population est essentielle pour la conservation des espèces surexploitées, telles que la morue franche (*Gadus morhua*). Nous avons comparé la variabilité génétique des microsatellites de populations de lacs de l'arctique canadien avec celles de morues habitant la baie de Gilbert et les côtes de l'Est canadien. Les populations arctiques possèdent la diversité génétique la plus faible et elles sont celles qui sont les plus structurées et distinctes du point de vue génétique. En revanche, les échantillons de l'Est canadien montrent une forte diversité allélique, ne sont pas significativement structurées génétiquement et ne se distinguent pas l'une de l'autre; les morues vivant dans la baie de Gilbert sont intermédiaires entre les groupes de l'arctique et de l'Est canadien. Nos résultats sont compatibles avec l'hypothèse selon laquelle les populations arctiques ont été établies il y entre 8000 et 5000 ans et qu'elles n'ont connu que peu ou pas de flux génétique depuis cette époque. Malgré leur isolement à l'extrême marge de la répartition de l'espèce, les populations arctiques ont conservé une forte hétérozygotie et des tailles de population génétique effective élevées par rapport aux tailles d'inventaire (rapports N_e–N_c). Les explications possibles de cette situation comprennent l'absence de pression de pêche qui permet la persistance de gros individus à forte fécondité, mais aussi des facteurs biotiques (par ex., l'absence de planctonophages) et abiotiques (par ex., la stochasticité environnementale faible) dans les lacs arctiques, ce qui minimise la variance individuelle du succès reproductif.

[Traduit par la Rédaction]

Introduction

The temporal stability and spatial scale of population structure is fundamental to our understanding of population dynamics and species' biology. In freshwater environments, dispersal barriers such as impassable river features or watershed separations can limit gene flow, thus generating high genetic variability and genetic distance among population units. By contrast, there are fewer barriers to dispersal in the marine environment, where it is generally believed that migration by various life-history stages of most marine species is too frequent and too extensive for significant genetic divergence to arise (Ward et al. 1994). Furthermore, factors such as natural selection and historical contact can upset the equilibrium between drift and gene flow, making allelic frequencies difficult to interpret for ma-

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rine fishes (Hilbish 1996). As a result, the genetic effects of isolation and small population size are largely unknown for most marine taxa. This issue is becoming increasingly important as global fisheries continue to deplete many species and populations are driven to unnaturally low numbers for extended periods of time, while remaining aggregations in geographically disjunct locales may experience limited gene flow. A well-known example of this is the Atlantic cod (*Gadus morhua*) in the Northwest Atlantic, where stocks have failed to recover from historically unprecedented declines despite a significant reduction in fishing pressure (Hutchings and Reynolds 2004).

Population structure of Atlantic cod

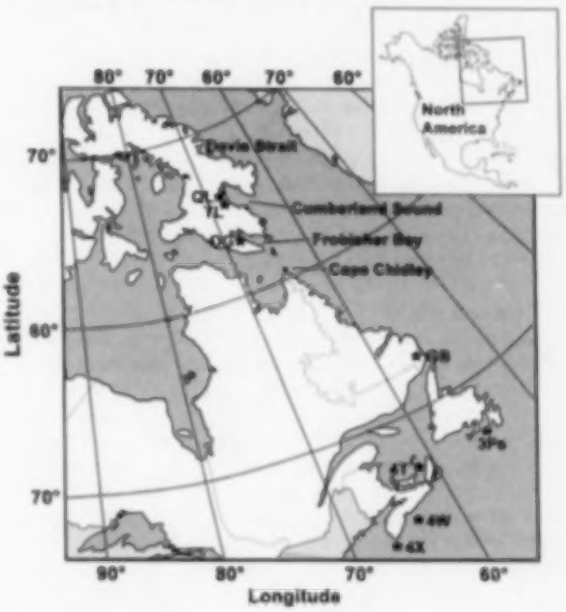
Evidence from vertebral data (Templeman 1981), geographic surveys (Hutchings et al. 1993), and tagging studies (Taggart et al. 1995) suggest that Atlantic cod spawning areas in the Northwest Atlantic are spatially discrete and temporally stable. The degree to which these aggregations represent reproductively isolated units has been intensely scrutinized. Studies using allozyme (e.g., Cross and Payne 1978) and mitochondrial DNA markers (e.g., Carr et al. 1995; Árnason et al. 2000) were unable to reject the null hypothesis of no genetic differentiation among putative populations of Northwest Atlantic cod. In contrast, the analysis of variation at highly polymorphic neutral microsatellite markers has revealed weak yet detectable spatial and temporally stable genetic differences between cod overwintering in inshore and offshore Newfoundland waters and between spawning complexes from various offshore regions (e.g., Bentzen et al. 1996; Ruzzante et al. 1996; Beacham et al. 2002). Spatial patterns of genetic differentiation among Northwest Atlantic cod populations are consistent with an isolation-by-distance model, whereby cod from more distant locations tend to be more genetically distinct (Pogson et al. 2001).

Ruzzante et al. (2000) compared microsatellite DNA variability in cod from Gilbert Bay, Labrador, with samples from Trinity Bay, Newfoundland, and the Northeast Newfoundland Shelf and found that samples from Gilbert Bay yielded distance measures 3- to 10-fold greater than analyses excluding Gilbert Bay. They concluded that genetically distinguishable coastal cod populations may exist or may have existed before the collapse of the northern cod fishery. Although Beacham et al. (2002) also found Gilbert Bay to be the most genetically distinct in their analysis of more than 5000 Northwest Atlantic cod, they found that annual variability within bays in northeastern Newfoundland was as great as variation among sites within any year, suggesting no evidence of bay-scale structure.

Marine-relict cod in Canadian Arctic lakes

Three marine-relict populations of Atlantic cod persist in saline coastal Arctic lakes on Baffin Island, Nunavut, Canada, at the northern extreme of the species' range in Canadian marine waters (Hardie and Hutchings 2003). Although Inuit Qaujimajatuqanginnut (traditional knowledge) of large cod in these lakes predates scientific reports, the cod population in Ogac Lake (OG), on the south shore of Frobisher Bay, has been known to science for only half a century (McLaren 1967; Patriquin 1967), and we have recently confirmed that two more populations, Qasigialiminiq (QL) and Tariujarusiq

Fig. 1. Sampling locations of 675 Atlantic cod (*Gadus morhua*) collected from Ogac (OG, $n = 68$), Qasigialiminiq (QL, $n = 105$), and Tariujarusiq (TL, $n = 98$) lakes on Baffin Island (Nunavut), Gilbert Bay (Labrador) (GB, $n = 100$), and Northwest Atlantic Fisheries Organization (NAFO) divisions 3PS ($n = 68$), 4T ($n = 93$), 4W ($n = 57$), and 4X ($n = 91$).



(TL), exist several hundred kilometres to the north, in the southwestern region of Cumberland Sound (Hardie and Hutchings 2003). The mitochondrial cytochrome *b* haplotypes of the cod in all three Arctic lakes are either the most common West Atlantic haplotype (haplotype A; sensu Carr et al. 1995) or single, third-position neutral mutations from this haplotype (haplotypes E and G; sensu Carr et al. 1995; D.C. Hardie and J.A. Hutchings, unpublished data). This supports a shared ancestry between the Arctic populations and progenitors of contemporary Northwest Atlantic cod and suggests that the use of a more rapidly evolving genetic marker is more likely to reveal the evolutionary history of these populations. Brooker (1994) used microsatellites to reveal low allelic richness and heterozygosity among cod from OG and found that they were significantly genetically distant from the two maritime Canadian cod stocks that were analyzed. Here, we extend the analysis using microsatellites to include two more Arctic populations, a bay-resident population, and several other eastern Canadian populations to consider the effects of isolation and colonization history on the genetic structure of the Arctic populations.

Currently, the northern limit of marine Atlantic cod populations occurs near Cape Chidley, at the northern tip of Labrador, and cod are absent from marine waters adjacent to the Arctic lakes (Fig. 1), which raises the questions of how and when these populations were colonized. The colonization and persistence of the Baffin Island populations depend on a suitable combination of (*i*) conditions that fostered a northward range expansion of the cod's range into Frobisher Bay and Cumberland Sound and (*ii*) appropriate changes in rela-

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tive sea level to create these coastal lakes during the same postglacial period. Because changes in the latitudinal range of Atlantic cod in response to changes in ocean temperatures are known to occur over relatively short time scales (e.g., Jensen 1948), it is plausible that Atlantic cod expanded their range northward during a period of ameliorated Arctic Ocean conditions associated with the collapse of the Laurentide ice sheet between 10 000 and 7000 years before present (ybp) (Dyke 1979; Aitken and Gilbert 1989; Stewart et al. 1993). Several lines of evidence suggest that this region of the Canadian Arctic has experienced a drop of about 70 m in relative sea levels as the result of glacioisostatic rebound over the past 9000 years and that the region is currently at or near pre-Holocene levels (references in Dyke 1979; Doner 2001). What is clear is that all three lakes derive from marine origins and were formed by glacioisostatic rebound over the last 9000 to 5000 years.

The present study

We employ genetic data from seven hypervariable microsatellite loci to elucidate the genetic characteristics of Atlantic cod populations in Arctic lakes at the northern extreme of the species' range compared with coastal and oceanic relatives. We test a hypothesis about the colonization history of these unique populations and use them as a microcosm of marine stocks to examine the genetic consequences of extended periods of isolation and low population size.

Elucidating the evolutionary history and genetic characteristics of these populations is interesting in its own right, as they have persisted for a long time in a highly unusual habitat at the extreme of the species' range. Furthermore, these isolated populations provide a unique opportunity to make inferences about the projected effects of extended periods of isolation at low population size on depleted cod stocks and other high-gene-flow marine species currently of conservation concern. The risk of loss of genetic diversity in stocks held at depressed population sizes for extended periods is heightened by the fact that genetic effective population size (N_e), a metric of the number of mature individuals that successfully contribute to subsequent generations, is invariably much lower than the census population size (N_c).

Our working hypothesis for the colonization history of the Arctic lacustrine populations, based on the geological record and paleobiological information, is that Atlantic cod colonized the lakes no more than 8000 ybp during a northward species' range expansion into Frobisher Bay and Cumberland Sound in response to a postglacial warming period. Aside from a probable period of low gene flow when cod were still present in the area and before the lakes had rebounded to sufficiently high levels to prevent migration, the populations would have been isolated from each other and from marine stocks since that time.

Materials and methods

Arctic study lakes

Ogac, Qasigialiminiq, and Tariujarusiq lakes are coastal meromictic lakes displaying strong vertical stability of thermal and salinity strata, which consist of a warmer, freshwater layer above a deeper saline layer, which itself overlies an anoxic hypersaline bottom layer. Stratification is maintained perennially by the influx of the highest spring tides, which reach 11.9 m in the vicinity of OG (Resor Island) and 6.9 m in the vicinity of QL and TL (Imigen Island), and breach the lake in monthly series during the open-water season (D.C. Hardie and J.A. Hutchings, unpublished data). The freshwater surface layer, which is deepest at the time of cod spawning (Patriquin 1967), may retain cod eggs in the lake, as they are negatively buoyant at low salinities. The lakes contain a simple community of marine organisms, which either persist as truly landlocked forms or are carried into the lake during tidal inflows. Atlantic cod are the only fish species present, and conspecifics make up a large part of the diets of adult cod, particularly of larger specimens (Patriquin 1967; D.C. Hardie and J.A. Hutchings, unpublished data). A resident cod population in Gilbert Bay (GB), Labrador, shares some features with the populations of these Arctic lakes, including strong thermal and salinity stratification and similar freshwater and tidal influences (Morris et al. 2002).

Sample collection

A total of 675 Atlantic cod were collected from eight sites (Fig. 1). Eastern Canadian samples were obtained from Fisheries and Oceans Canada trawl surveys of North Atlantic Fishery Organization (NAFO) divisions 3Ps, 4T, 4W, and 4X in 2002. Gilbert Bay samples were collected by hook and line in 2004 (C.J. Morris, Science, Oceans and Environment Branch, Department of Fisheries and Oceans, St. John's, Newfoundland and Labrador). Samples from Arctic lakes were collected using minnow traps, gill nets, and hook and line in 2003 and 2004.

Microsatellite DNA

Genomic DNA was extracted from tissue stored in 95% ethanol, using the DNEasy Tissue Kit following manufacturer's instructions (Cat. No. 69504; QIAGEN Inc., Mississauga, Ontario, Canada). Six individuals were duplicated in each 96-well extraction plate to ensure consistent scoring of microsatellite alleles. The following seven microsatellite loci were amplified by polymerase chain reaction (PCR) using a PTC-200 thermal cycler (MJ Research PTC-0200; Bio-Rad Laboratories Ltd., Mississauga, Ontario, Canada) and following conditions specified by the following authors: *Gmo3, Gmo8, Gmo19, Gmo34, Gmo35* (Miller et al. 2000), *Tch5* (O'Reilly et al. 2000), and *Mae9* (O'Reilly et al. 2002). Reactions were carried out in 10 µL volumes containing 50 mmol·L^{-1} KCl, 20 mmol·L^{-1} Tris (pH 8.4), 0.2 mmol·L^{-1} of each dNTP, 0.5 mmol·L^{-1} of each Hex-labelled primer, 0.8 µL *Taq* DNA polymerase, and 20–100 ng of template DNA. Following PCR, 3 µL of product were added to 8 µL of deionized formamide (5% *w/v* bromophenol blue), denatured for 10 min at 95 °C, and size-fractioned on a 0.4 mm, 6 mol·L^{-1}, 6% denaturing polyacrylamide gel for 1–2 h (depending on the size of the expected product) alongside a 4 bp allelic ladder constructed out of genomic DNA (run next to a commercial 60–400 bp fluorescent (CRX) ladder (Cat. No DG6221; Promega, Madison, Wisconsin)). Ladder and microsatellite alleles were visualized using an FMBIO II® florescent imaging system (Hitachi Software Engineering America Ltd., San Francisco, California). The six duplicated samples from each extraction plate were scored blind and checked against each other for consistency. All gels were

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scored by two individuals and either run again (28 individuals at various loci) or excluded from analysis (three individuals were excluded at all loci) if there were any inconsistencies.

Statistical analysis

Microsatellite diversity within each sample was tested for observed (H_O) and expected (H_E) heterozygosities, Hardy–Weinberg equilibrium (HWE) for each locus in each population (probability test), and evidence of linkage disequilibrium, using GENEPOP 3.4 (Raymond and Rousset 1995). Mean allelic richness (A) was corrected for a minimum sample size of 57 (A_c), using the rarefaction method of Goudet (2001), and compared using analysis of variance (ANOVA; Analyse-It Software Ltd., Leeds, UK). Allele distributions were compared, and global and pairwise variance in microsatellite allele identity (F_{st}; Wright 1951) and size (R_{st}; Slatkin 1995) were estimated using GENEPOP 3.4. Significance tests for F_{st} were performed by bootstrapping across loci 1000 times per individual comparison (Goudet 2001).

We compared variance in R_{st} and F_{st} to test the relative importance of mutation (R_{st}) and drift (F_{st}) for population differentiation, using SPAGeDi 1.1 (Hardy and Vekemens 2002) following Hardy et al. (2003). This test does not rely on assumptions of constant population size or mutation–drift equilibrium and is expected to remain exact even when these assumptions are violated. Although the test is sensitive to nonneutrality of loci if different alleles size ranges are selected for in different populations, it is robust if there is selection for the same range of allele sizes across the species' range (Hardy et al. 2003). This approach has been used in other studies of north temperate fishes to infer dynamics of colonization history (e.g., Fraser and Bernatchez 2005). We tested the null hypothesis $R_{st} = F_{st}$ (i.e., differentiation is driven mainly by drift, thus F_{st} is more appropriate) and the alternative hypothesis $R_{st} > F_{st}$ (i.e., differentiation is driven mainly by stepwise mutational model (SMM) like mutations, thus R_{st} is more appropriate), using a permutation test of allele sizes (5000 random permutations) to generate a simulated distribution of R_{st} values. Nei's (1978) unbiased genetic distances (D) among sampling locations were calculated and tested for significance using a permutation test with 1000 iterations of the data (Genetix Version 4.05 for Windows; Belkhir et al. 1996). The sequential Bonferroni correction was applied to correct for x simultaneous tests where appropriate ($\alpha = 0.05/x$) (Rice 1989).

Testing models of colonization history and genetic differentiation

We used EasyPop (Balloux 2001) to simulate the colonization history of the Arctic populations in order to test which combinations of genetic effective population size (N_e) and migration rate (m) could explain observed among-population variation in F_{st}, observed heterozygosity (H_O), and allelic richness (A). Unfortunately, N_e must be held constant throughout simulations performed using EasyPop, so it is not possible to simulate population growth after a founder event by a smaller number of individuals. Several other user-selected parameters are required, for which we selected, as a priority, the most realistic values, and secondarily, values towards the "conservative" end of the range of realistic options (i.e., those most likely to allow the populations to reach values of the genetic parameters described above). We used generation times (G) of 7 years, which is the mean age of mature cod from 50 samples from each of the three Arctic lakes, and 12 years, which is the generation time of the northernmost cod stocks for which data are available to obtain these estimates (Hutchings 1999). We used a colonization time of 8000 ybp (667 and 1143 generations for G = 7 and G = 12, respectively) based on the most conservative (oldest) period when Atlantic cod are likely to have colonized the lakes. We applied the island model of migration and allowed free recombination, as these were the most realistic descriptors of the colonization of these lakes. We used a microsatellite mutation rate of 0.0005, which is at the conservative end of the standard microsatellite mutation rate range suggested by Jarne and Lagoda (1996). We adopted the mean allelic richness across loci (17 possible allelic states) from eastern Canadian population groups from the current study and allowed maximal initial population variation as we found no evidence of a recent population bottleneck (see below). We selected a mixed mutation model, which tends to fit microsatellite data sets better than other options (Di Rienzo et al. 1994), at a proportion of 90% SMM – 10% KAM (K-allele model), also recommended as providing the best fit for microsatellite data (Cornuet and Luikart 1996).

We used BOTTLENECK 1.2.02 (Cornuet and Luikart 1996) to test for evidence of a recent reduction in population size in the three Arctic populations. This program compares observed with expected gene diversity, which is computed from the observed number of alleles given the sample size under the assumption of mutation–drift equilibrium. Populations having recently experienced a reduction in effective population size exhibit a correlative reduction in allele number and gene diversity. Because the former is reduced faster than the latter, observed gene diversity in recently bottlenecked populations tends to be higher than expected equilibrium gene diversity (Luikart et al. 1999). This is tested using the qualitative sign and Wilcoxon tests of whether populations show a significant number of loci with heterozygosity excess and the quantitative mode-shift test of allele frequency distributions. The latter tests whether the allele distribution is approximately L-shaped (as expected under mutation–drift equilibrium) or not (recently bottlenecked populations are mode-shifted). We used 5000 iterations of the two-phase model (TPM), consisting mostly (90%) of one-step changes but allowing 10% multistep changes, as recommended for microsatellite data (Cornuet and Luikart 1996), with 30% variance for the TPM.

Results

Microsatellite DNA

The number of alleles per locus varied from 1 to 12 in the three Arctic populations, from 2 to 24 in Gilbert Bay, and from 2 to 45 in the eastern Canadian populations (Table 1). There was a significant difference in mean corrected allelic richness among the Arctic populations (4.7–6.4), Gilbert Bay (10.1), and the eastern Canadian populations (14.9–16.4) (ANOVA, $P = 0.04$). Pairwise linkage disequilibrium analysis (Fisher's method) verified that all loci were independent across all populations ($\alpha = 0.05/x$). The only populations that were not in Hardy–Weinberg equilibrium (HWE) were OG

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Table 1. Per-locus total allelic richness, allelic richness corrected to a sample size of 57 individuals (in parentheses), mean total allelic richness ($\bar{A}$), and mean corrected allelic richness ($\bar{A}_c$) for Atlantic cod (*Gadus morhua*) populations from Arctic lakes on Baffin Island, Nunavut (OG, QL, TL), Gilbert Bay, Labrador (GB), and Northwest Atlantic Fisheries Organization (NAFO) divisions 3PS, 4T, 4W, and 4X.

	N	*Gmo3*	*Gmo8*	*Gmo19*	*Gmo34*	*Gmo35*	*Mae9*	*Tch5*	$\bar{A}$ ($\bar{A}_c$)
OG	68	3 (3)	10 (9.6)	6 (6)	2 (2)	5 (5)	7 (6.8)	9 (8.9)	5.9 (5.7)
QL	105	3 (3)	9 (8.1)	7 (6.8)	3 (2.9)	6 (6)	12 (11.9)	9 (8.8)	6.8 (6.4)
TL	93	3 (2.9)	6 (5.6)	6 (5.9)	1 (1)	6 (5.9)	7 (7)	6 (5.6)	4.8 (4.7)
GB	100	2 (2)	14 (12.8)	16 (14.2)	5 (5)	6 (5.9)	24 (20)	11 (10)	10.0 (9.1)
3PS	68	5 (4.7)	21 (20.4)	25 (23.6)	5 (4.8)	7 (7)	43 (39.7)	17 (16.7)	16.7(14.9)
4T	93	5 (3.8)	25 (20.8)	27 (23.7)	5 (5.0)	7 (6.6)	45 (37.2)	21 (19.4)	16.7(14.9)
4W	57	3	18	23	6	7	42	19	16.9
4X	91	5 (4.5)	20 (17.8)	25 (22.5)	5 (4.6)	7 (6.6)	45 (38.3)	20 (18.5)	16.1(14.6)
$\hat{A}$		8	37	38	6	7	67	24	

Table 2. F_{IS} values (inbreeding coefficient; Weir and Cockeram 1984), *p* values for the probability test for Hardy–Weinberg equilibrium across each locus and population, and expected (H_E) and observed (H_O) heterozygosity for seven microsatellite loci from eight populations of Atlantic cod (*Gadus morhua*).

	Gmo3	*Gmo8*	*Gmo19*	*Gmo34*	*Gmo35*	*Mae9*	*Tch5*	*p* value	H_E	H_O
OG	–0.017	–0.001	0.019	0.151	0.113*	–0.086	–0.101	0.04	0.60	0.60
QL	–0.168	–0.040	0.007	–0.129	–0.036	0.003	0.052	0.62	0.70	0.72
TL	–0.042	–0.081	0.156*	fixed	–0.099	–0.090	0.474*	<0.01	0.60	0.56
GB	–0.016	0.028	0.048	–0.017	–0.065	0.029	0.080	0.81	0.70	0.69
3PS	0.038	0.003	–0.041	–0.021	–0.009	0.047	0.089	0.59	0.76	0.75
4T	–0.088	–0.028	–0.042	–0.007	0.038	0.034	–0.020	0.36	0.77	0.77
4W	0.101	–0.008	0.041	–0.019	0.022	0.008	–0.022	0.66	0.78	0.78
4X	–0.037	–0.045	0.043	–0.049	–0.056	0.080*	0.011	0.59	0.77	0.77
p value	0.899	0.239	0.129	0.562	0.282	0.387	<0.01			
H_E	0.33	0.82	0.85	0.54	0.73	0.86	0.85			
H_O	0.34	0.84	0.82	0.55	0.73	0.85	0.80			

Note: Significant F_{IS} values ($\alpha = 0.05$, adjusted for multiple comparisons) are indicated by an asterisk.

and TL. These two populations showed the only per-locus deviations from HWE, which occurred at *Gmo35* in OG and at *Tch5* and *Gmo34* (fixed for one allele) in TL (Table 2; Fig. 2).

Many alleles present in the eastern Canadian populations were absent from the Arctic populations and, to a lesser degree, from the Gilbert Bay population (Fig. 2). There was no significant difference ($\alpha = 0.05/x$) in the frequency distribution of alleles between any of the eastern Canadian populations at any loci. In contrast, the frequency distribution of alleles was unique at every locus within each Arctic population, with the exception of one comparison between TL and GB at the *Gmo3* locus. The frequency distribution of alleles for cod from Gilbert Bay was unique at all loci except *Gmo3* and *Gmo35*, for which the distributions did not differ significantly from the eastern Canadian populations. There were three unique alleles present at *Gmo8* in the OG population.

There was high variance in allelic identity among the Arctic populations ($F_{st} = 0.20$–0.23). Variance was also high between any of the Arctic populations and Gilbert Bay ($F_{st} = 0.16$–0.21) and between any of the Arctic populations and any of the eastern Canadian populations ($F_{st} = 0.08$–0.14). Variance was lower between Gilbert Bay and any of the eastern Canadian populations ($F_{st} = 0.05$–0.06), and there was no significant variance in allelic identity among any of the eastern Canadian populations ($F_{st} < 0.003$) (Table 3).

We were unable to reject the null hypothesis (H_0: $R_{st} = F_{st}$) used to assess the relative importance of mutation (R_{st}) and drift (F_{st}) for population differentiation (global $p > 0.3$, per-locus $p > 0.3$ at all loci except *Gmo19* where $p = 0.06$). This suggested that allele-identity-based measures of genetic structure and distance were more appropriate and led to the use of F_{st} and Nei's (1978) unbiased genetic distance (D).

The Arctic populations were significantly genetically distant from each other ($D = 0.484$–0.596) and from Gilbert Bay samples ($D = 0.527$–0.611) and less distant from the eastern Canadian samples ($D = 0.242$–0.395) at the 5% significance level after correction for multiple comparisons (Table 3). The samples from Gilbert Bay were also significantly genetically distant from eastern Canadian samples, although only moderately so ($D = 0.156$–0.177) relative to comparisons involving Arctic populations. There was no significant evidence of genetic distance among groups of eastern Canadian samples.

Effective population size (N_e) of Arctic populations

Simulating the isolation of the three Arctic populations showed that even under the most conservative scenario of

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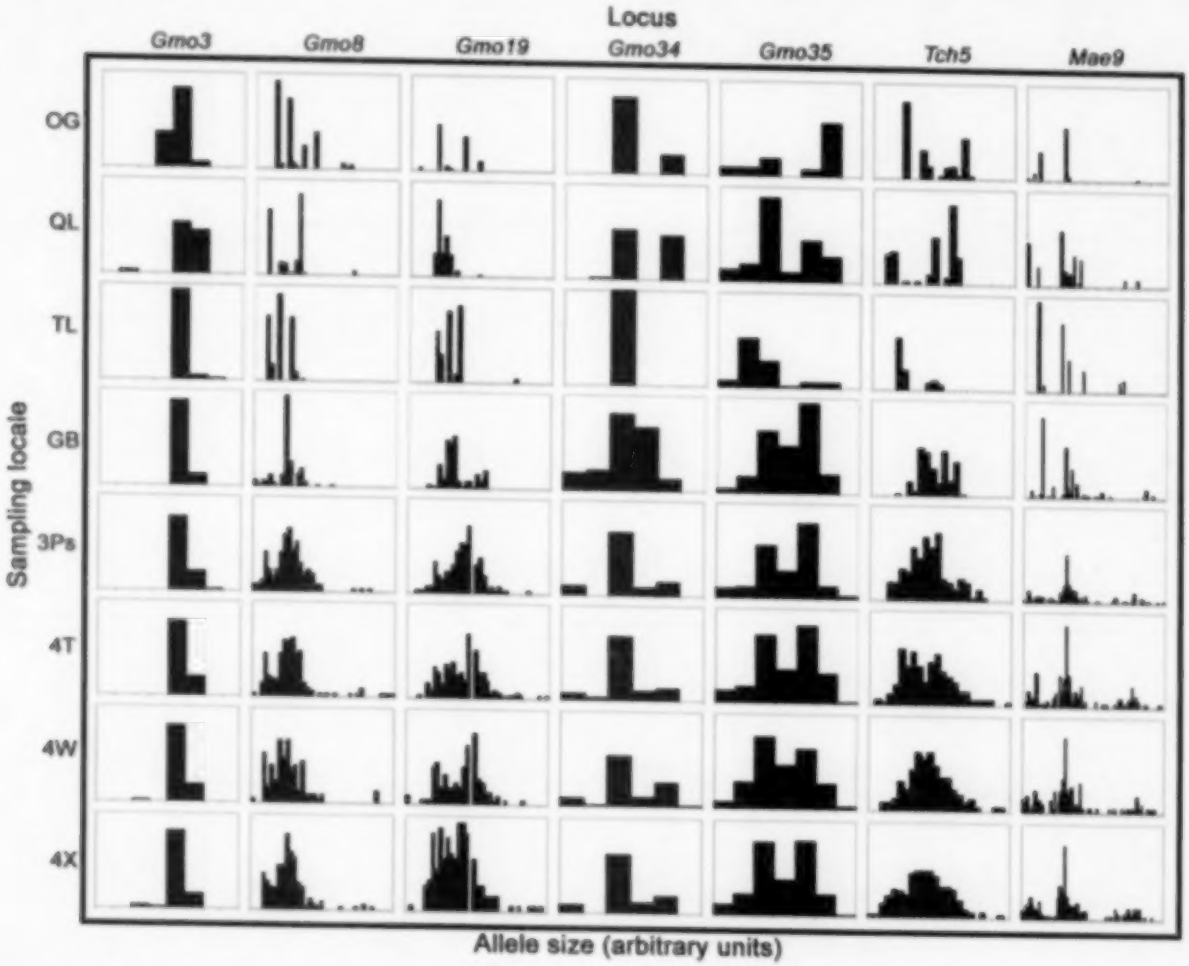
Fig. 2. Frequency distributions of allele sizes at seven microsatellite loci for Atlantic cod (*Gadus morhua*) populations from Arctic lakes on Baffin Island, Nunavut (OG, QL, TL), Gilbert Bay, Labrador (GB), and Northwest Atlantic Fisheries Organization (NAFO) divisions 3PS, 4T, 4W, and 4X.



initial conditions, genetic effective population size must have been in the range of 300–1000, with no or extremely little migration over the last 8000 years, in order to account for the high levels of observed variance in allelic identity (Table 4). For example, simulations based on the most conservative model parameters (high number of generations (1143), low founding allelic richness, low mutation rate) suggest that a sustained N_e of 1000 with no migration for 1143 generations (8000 years) would have been necessary to generate the observed global F_{st} values of 0.22. All else being equal, simulating even very low amounts of migration (0.001) requires an N_e of 300 in order to simulate $F_{st} = 0.22$ for either generation time.

For illustrative purposes, the equation

$$(1) \qquad H_E = 4N_e\mu/(4N_e\mu + 1)$$

shows that low heterozygosity infers a small effective population size. Based on eq. 1, the actual ($\hat{H}_E = 0.63$; Table 2) and modelled ($\hat{H} = 0.66$; Table 4) heterozygosities yield N_e values of 850 and 975, respectively, which are similar to the most parsimonious estimate of N_e from our model (1000) for the shorter generation time.

There was no evidence of a recent reduction in population size based on either quantitative (sign test, $p > 0.35$; Wilcoxon test, $p > 0.37$) or qualitative (mode-shift test, normal L-shaped allele frequency distributions) analyses in any of the Arctic populations.

Discussion

Genetic consequences of extended periods of small population size

Perhaps the most unambiguous component of our analysis is that the Arctic populations of Atlantic cod are the most strongly genetically structured, the most genetically distinct, have the lowest allelic richness, and possess the most unique

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Table 3. Multilocus variance in allelic identity as F_{st} (bottom half of matrix; Wright 1951) and Nei's (1978) unbiased genetic distance (top half of matrix) with significant values ($\alpha = 0.05$) adjusted for multiple comparisons indicated by asterisk.

	OG	QL	TL	GB	3PS	4T	4W	4X
OG		0.596*	0.484*	0.611*	0.395*	0.355*	0.379*	0.362*
QL	0.1952*		0.565*	0.572*	0.322*	0.312*	0.269*	0.280*
TL	0.2381*	0.2234*		0.527*	0.274*	0.242*	0.275*	0.251*
GB	0.1966*	0.1563*	0.2141*		0.177*	0.173*	0.161*	0.156*
3PS	0.1407*	0.0943*	0.1432*	0.0576*		–0.003	0.008	–0.003
4T	0.1291*	0.0913*	0.1295*	0.0558*	–0.0008		0.008	0.001
4W	0.1359*	0.0806*	0.1456*	0.0526*	0.0025	0.0026		–0.002
4X	0.1299*	0.0834*	0.1324*	0.0510*	–0.0008	0.0004	–0.0005	

Table 4. Initial input variables for population divergence simulations using the program EasyPop (Balloux 2001) and resulting final allelic richness, heterozygosity, and global F_{st} predictions.

Genetic effective population size (N_e)	Migration (m)	Generations since isolation	Final allelic richness (observed 6)	Final heterozygosity (observed 0.63)	Final F_{st} (observed 0.22)
500	0	667	11	0.55	0.31
300	**0.001**	**667**	**8**	**0.60**	**0.22**
750	**0**	**667**	**14**	**0.67**	**0.21**
1000	0	667	14	0.71	0.16
750	0	1143	13	0.60	0.27
300	**0.001**	**1143**	**8**	**0.52**	**0.21**
750	0.001	1143	13	0.75	0.10
1000	**0**	**1143**	**14**	**0.66**	**0.22**
1250	0	1143	15	0.71	0.18

Note: Combinations of input parameters yielding the closest predictions of allelic richness, heterozygosity, and F_{st} to observed population values given in bold.

allele distributions. The eastern Canadian populations fall at the opposite extreme, and cod from Gilbert Bay are intermediate for all of the above genetic characteristics, as expected based on previous research (Ruzzante et al. 2000).

We expected an increase in allelic richness from the small, isolated populations in the Arctic lakes to the larger resident population in Gilbert Bay to the large, admixed eastern Canadian populations. Allelic richness is greater at any one time and new alleles persist for longer periods in larger populations because average time between mutation and fixation of new alleles is proportional to N_e (Beebee and Rowe 2004). The probability of the loss of rare alleles as the result of chance events is greater in smaller populations, which increases their vulnerability to factors such as inbreeding depression. The presence of three unique alleles in OG is intriguing and reinforces that this population has been isolated for a sufficient period for new mutations to become established. The trend of increasing allelic richness from Arctic (4.7–6.4) to Gilbert Bay (10.1) to eastern Canadian (14.9–16.4) populations is consistent with the hypothesis of increasing gene flow and effective population size across the three groups. This supports the hypothesis that the Arctic populations are comparatively small and have been isolated for a long time and is consistent with the low microsatellite allelic richness previously reported among cod from Ogac Lake (Brooker 1994). The moderate allelic richness of the Gilbert Bay population supports the hypothesis that oceanographic features act to retain early life history stages within the bay and that adult cod in this population are philopatric (Morris et al. 2002), although population size and gene flow are higher than in the Arctic lakes. The high allelic richness in the eastern Canadian populations is consistent with the high dispersal and high effective population size model that is assumed for many marine species.

The result that OG and TL are out of Hardy–Weinberg equilibrium (HWE) is most likely due to single loci being out of HWE in each population (*Gmo35* in OG, *Tch5* in TL). The low values of $\bar{A}_L$ and H_E for TL and the fact this population was not in HWE are most likely due to the fixation of *Gmo34* for one allele in this population. TL also had the lowest population size of the three Arctic lakes (D.C. Hardie, unpublished data), thus having a higher probability of inbreeding, which may have contributed to a loss of heterozygosity. Overall, the results suggest that even if the Arctic populations experienced an early colonization bottleneck, population growth with approximately random mating has restored Hardy–Weinberg proportions at most loci. The lack of evidence for recent reductions in population sizes in the Arctic lakes further supports this, suggesting that population dynamics have been historically stable.

Genetic differentiation among populations

Genetic differentiation based on both allele identity (F_{st}) and size (R_{st}) was approximately two orders of magnitude higher for the Arctic populations than has previously been reported for the species (Ruzzante et al. 1999; Beacham et al. 2002). Although the values reported here are extremely high for cod, and for any marine species, they are not particularly unusual for freshwater fishes, for which measures of genetic structure of this magnitude and greater are common. The lack of evidence of SMM-like processes contributing to population differentiation suggests that the random loss of

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alleles by genetic drift has been more important than mutation over the circa 1000 generations since the colonization of the Arctic lakes and accounts for most of the strong genetic structure among these populations and between each of them and the Gilbert Bay and eastern Canadian populations. Furthermore, the observation that the Arctic populations all show depressed allelic richness and unique allele frequency distributions provides further evidence of drift-related processes rather than mutation. These results are consistent with low effective population size in the Arctic populations, a situation in which drift-related processes would be expected to exceed mutation, especially over a relatively short evolutionary period. However, founder effects cannot be discounted as an explanation for lower allelic richness among the Arctic populations, although our results suggest that these events, if they did happen, have not been recent. However, it is plausible that a bottleneck occurred during colonization, such that some alleles from the founding population never made it into the Arctic lakes, and that evidence of this bottleneck was erased with the reaching of a new equilibrium between drift and mutation at the new N_e (approximately 0.2–4.0 founder-N_e generations; Luikart and Cornuet 1998). The range of genetic distances reported for any comparisons involving the Arctic populations are consistent with a previous comparison between OG and maritime cod samples (D = 0.475; Brooker 1994).

Colonization history: evidence for a high N_e–N_c ratio for a marine fish

Our results are consistent with the hypothesis that the Arctic populations were colonized by Northwest Atlantic cod, which could have expanded their range northward during a period of ameliorated Arctic Ocean conditions between 8000 and 5000 ybp. Since then, colder temperatures and increased ice cover likely acted to push Atlantic cod out of Canadian Arctic waters, except in the warmer saline lakes formed by glacioisostatic rebound during this same period where these unique populations have persisted in isolation from each other and from marine stocks. Small population size and the absence of migration set up an ideal situation for genetic drift to strongly alter genetic diversity, even on relatively short evolutionary time scales. This random loss of alleles, to the point of fixation in one instance, may have contributed to the genetic distinctiveness of the Arctic populations by several different metrics examined in this study, although founder effects could have contributed to their low allelic richness.

A further test of our colonization hypothesis was to model combinations of population size and migration rate to reveal whether the observed F_{st}, heterozygosity, and allelic richness values from the present study are consistent with our hypothesised divergence time of 5000 to 8000 ybp. Simulations based on colonization 8000 ybp suggest that a sustained N_e in the range of 300–1000 (depending on migration and generation time) would be necessary to attain observed values of heterozygosity and F_{st}, and calculations based on observed and modelled heterozygosities suggest a value of N_e closer to the upper end of this range, 850–975. Although the assumption that N_e remained constant throughout colonization may be somewhat unrealistic, our results suggest that the sizes of the Arctic populations have been stable for a long period. This result, coupled with the relatively high heterozygosities in the Arctic populations, is interesting from a conservation perspective, as it suggests that they may be genetically predisposed to be able to tolerate extended periods of low abundance and high inbreeding without suffering severe demographic or evolutionary consequences. Considering Patriquin's (1967) estimate that there were on the order of 10 000 cod greater than 25 cm long in the Ogac Lake population, the N_e–N_c ratio would have to be between 3×10^{-2} and 10^{-1}, which is orders of magnitude greater than the values suggested for marine fishes (Hutchings and Reynolds 2004), including Atlantic cod (Hutchinson et al. 2003), which range from 10^{-3} to 10^{-5}. Given the depauperate fauna in the lakes, 10 000 cod per lake appears a generous estimate, and it is unlikely that census size greatly exceeds this amount in any of the Arctic lakes (D.C. Hardie and J.A. Hutchings, unpublished data). As such, it appears that the N_e–N_c ratio may be higher, even much higher, in the Arctic populations than previously estimated for cod and other marine fishes.

Given that a higher N_c is unlikely, additional factors that could increase the N_e–N_c ratio are higher average individual reproductive success and (or) lower individual variability in reproductive success (Lande and Barrowclough 1987). There are several features of the Arctic populations, relative to oceanic stocks, that could act to decrease individual variability in reproductive success. First, the perennial stratification of the lakes provides a retention mechanism for cod eggs and larvae, which are negatively buoyant at low salinity and as a result are held in the deeper, stable, saline layer. The restricted size and highly stable nature of the saline layers may mean that recruits are not lost from the population to the same degree that oceanographic features such as storms, currents, and other stochastic events remove recruits from marine populations. Furthermore, these factors may help to ensure that spawning is unlikely to be prevented, interrupted, or otherwise affected unduly by environmental forces. At first consideration, the absence of other fish species in Arctic lakes would appear to be deleterious to fitness, forcing cod to resort to cannibalism or to forage on unusual prey such as sea urchins (*Strongylocentrotus droebachiensis*) and bivalves. However, compared with oceanic environments where larval predators such as herring (*Clupea harengus*) and mackerel (*Scomber scombrus*) are abundant, the absence of any planktivorous fish species in the Arctic lakes may act to increase the number of recruits per spawner and lower individual variability in reproductive success. Larval predation is one of the greatest sources of mortality in marine fishes, and the contemporary increase in planktivorous fish stocks has been invoked as a possible mechanism contributing to the failure of depleted cod stocks to recover (Swain and Sinclair 2000; Walters and Kitchell 2001). In essence, these factors may act to dampen the "sweepstakes" effect thought to account for high variability in reproductive success and low N_e–N_c ratios in the stochastic marine environment (Hedgecock 1994). It may be possible to test these speculations by assessing genetic variability within larval cohorts, using a paternity assignment program such as Parentage (Emery et. al 2001).

Despite features that may contribute to the maintenance of a high N_e–N_c ratio, the persistence of Arctic populations at the extreme of the species' range, at low temperatures, in

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depressed and variable salinity, under extended periods of ice cover, and among a sparse and depauperate fauna is remarkable. Although we present evidence of strong genetic structure and distinctiveness in these populations, they are in HWE and maintain reasonably high levels of heterozygosity, despite the relatively strong effect of drift or founder effects on allelic richness and allelic distributions. In addition to decreased individual variability in reproductive success via the mechanisms outlined above, two other related factors (the absence of fishing and large body size) may help to explain how these unlikely populations have persisted despite the expected genetic consequences of extended periods at low population size. Although marine cod approached 100 kg in size in the past, such individual sizes are unheard of today. Indeed, Atlantic cod of the size class observed in the Arctic lakes, up to 157 cm, are extremely rare in contemporary marine populations. Although the absence of large size classes in harvested fisheries is not uncommon, a much more disquieting phenomenon may be at play whereby fishing acts as an intense form of size-selective mortality, leading to evolutionary changes in the size structure of harvested species (Law 2000; Olsen et al. 2004). This too has been proposed as a factor contributing to the nonrecovery of collapsed marine fishes, especially for highly fecund "lottery" spawners, which rely on the strong relationship between body size and fecundity to ensure recruitment in the face of intense juvenile mortality (Hutchings 2005). It is possible that the absence of fishing in the remote Arctic populations has resulted in a large average size and high fecundity, which may combine with low variability in reproductive success in these sheltered lakes to ensure sufficient recruitment in these ostensibly difficult settings.

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Olfactory inhibition and recovery in chum salmon (*Oncorhynchus keta*) following copper exposure

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Abstract: Copper can be an effective hatchery treatment to control certain parasitic and fungal infections in salmonids, yet it is also highly toxic to the fish's olfactory system. Numerous reports have described the early inhibitory impacts of copper on fish olfaction, but very few have addressed processes of recovery. Here we show that the chum salmon (*Oncorhynchus keta*) olfactory system can recover from short-term (4 h) exposures to copper (3–58 μg·L^{-1}) within 1 day, as indicated by electro-olfactogram (EOG) recordings. Recovery followed initial EOG reductions of up to 88%. We also applied a novel cytophysiological approach to monitor changes in the olfactory receptor cells using a fluorescent styryl dye, AM1-43. Copper's influence on the number of receptor cells that labelled with the dye was biphasic, initially increasing the number at the two lowest copper concentrations (3 and 8 μg·L^{-1}) and decreasing the number at the two highest concentrations (24 and 58 μg·L^{-1}). Changes in the ability of olfactory receptor neurons to internalize the dye were monitored up to 10 days in fish recovering from copper exposure.

Résumé : Le cuivre peut servir de traitement efficace des pisciculture afin de contrôler certaines infections à parasites ou à champignons chez les salmonidés, bien qu'il soit très toxique pour le système olfactif des poissons. De nombreux travaux ont décrit les premiers effets inhibiteurs du cuivre sur l'olfaction des poissons, mais très peu se sont intéressés aux processus de récupération. Nous montrons ici que le système olfactif du saumon kéta (*Oncorhynchus keta*) peut récupérer après de courtes (4 h) expositions au cuivre (3–58 μg·L^{-1}) en moins d'une journée, d'après les enregistrements d'électro-olfactogrammes (EOG). La récupération fait suite à des réductions initiales de l'EOG pouvant atteindre 88 %. Une nouvelle méthode cytophysiologique utilisant un colorant styryle fluorescent, AM1-43, permet de suivre les changements dans les cellules olfactives réceptrices. L'effet du cuivre sur le nombre de cellules réceptrices marquées par le colorant se fait en deux phases, au départ une augmentation du nombre aux deux concentrations les plus faibles de cuivre (3 et 8 μg·L^{-1}) et une diminution aux deux concentrations les plus élevées (24 et 58 μg·L^{-1}). Nous avons suivi les changements de la capacité des neurones olfactifs récepteurs d'incorporer le colorant jusqu'à 10 jours chez des poissons en train de récupérer d'une exposition au cuivre.

[Traduit par la Rédaction]

Introduction

Short-term exposures to dissolved copper (on the order of minutes to hours) and at relatively low concentrations (1–20 μg·L^{-1}) can inhibit the electrical properties of olfactory receptor neurons in salmon (Baldwin et al. 2003; Sandahl et al. 2004). Copper targets several sites of the olfactory neuron, and depending on the conditions of exposure, inhibitory effects can be either reversible or irreversible (Moran et al. 1992; Julliard et al. 1996; Hansen et al. 1999). Irreversible damage caused by high or prolonged exposures to copper can target cellular surface proteins, membrane structure, or internal organelles and can eventually lead to cell death (Brown et al. 1982). Subsequent regeneration of the neurons may require several weeks. Less attention, however, has been given to the reversible effects of copper on the fish olfactory system and the time required for recovery, following relatively brief or low concentration exposures.

In urban and agricultural environments, exposures to copper can occur when storm water pulses into an aquatic habitat (Anderson et al. 1996; Soller et al. 2005; Wheeler et al. 2005). In a hatchery setting, fish can be intentionally treated with copper to control disease (e.g., Schlenk et al. 1998). Olfaction plays an essential role in the survival of salmon, and the olfactory nervous system is highly sensitive to the impacts of surface water toxicants, such as copper. Odorant cues convey important information about the aquatic environment, including the chemical constituents of natal habitat, habitat quality, the presence and location of food, conspecifics and predators, and the reproductive status of spawning partners. It is therefore important that brief copper exposures to salmon, either as pulses into the environment or

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as treatments by hatchery managers, do not cause long-lasting olfactory impairment.

The electro-olfactogram (EOG) has been one of the primary tools for monitoring the inhibitory effects of toxicants on electrical field potentials of olfactory receptor neurons in fish (e.g., Hara et al. 1976). Upon binding of odorant agonists, populations of G protein-coupled receptors (GPCR) transduce stimuli information to second messengers within the neuron, creating a generator potential that triggers an action potential (Ferguson 2001). An electrode positioned above the surface of the olfactory epithelium measures the summated electrical responses from stimulated neurons and is recorded as the EOG (Hara 1992).

The EOG has been useful in studying the effects of contaminants on primary sensory neurons of the olfactory system, particularly in fish (Baatrup et al. 1990; Jarrard et al. 2004; Sandahl et al. 2004). Heavy metals, for example, can inhibit the electrical properties of olfactory neurons, presumably by blocking ligand-gated and (or) voltage-gated ion channels. This inhibitory effect can be reversible, as indicated by time-course EOG recordings in salmon (Baldwin et al. 2003). However, more severe, irreversible damage to a neuron ultrastructure by metals, including the GPCR-rich cilia and microvilli, the cellular membrane, and supportive organelles, can result in extended reductions in neuronal electrical activity.

Few data are available describing the subtle impacts that heavy metals and other contaminants can have on the complex mechanics of the olfactory process. Toxins can target a number of sites along the agonist–transduction–messenger–electrical signaling pathway, ultimately resulting in inhibitory, excitatory, or distorted responses reaching the olfactory bulb (Horning and Trombley 2001; Sandahl et al. 2004). Along with ion channels as initial targets of heavy metals, odorant-binding GPCRs are also susceptible sites. Metals, like copper, may act as odorant agonists, alter a receptor protein's steric properties, alter transduction or second messenger pathways, or affect processes involving GPCR endocytosis and recycling. Although few studies have addressed this area of metal toxicity in the olfactory system, related research may provide useful tools to begin.

Fluorescent styryl dyes, such as AM1-43 and similar dyes, have been used to investigate synaptic activity at neuron terminals (Pyle et al. 1999; Cousin and Robinson 2000; Li et al. 2003). In these previous studies, the styryl dyes were shown to enter active neurons via endocytosis following nerve stimulation. Although there are no synaptic connections at the apical surface of olfactory receptor neurons — the initial target site of copper toxicity — we speculated that similar endocytic processes of the olfactory receptor neuron would also allow the dye to penetrate an active cell.

In the present study, we combined the two techniques, the EOG and AM1-43 labelling of olfactory receptor neurons in chum salmon (*Oncorhynchus keta*), to address a practical problem faced by hatchery managers in northern Japan. In Japanese aquaculture, treatment of fungal and parasitic infections in salmonids has often been carried out using the chemical agent, formalin. Because of its carcinogenic risks, the use of formalin at Japanese hatcheries has mostly been eliminated. At the Hokkaido Fish Hatchery (Eniwa, Japan), preliminary experiments have demonstrated that copper treatments can reduce infections caused by the fungus *Saprolegnia* in chum salmon roe and reduce the parasite *Ichthyobodo necator* in chum and masu salmon (i.e., cherry salmon, *Oncorhynchus masou masou*) fry (N. Koide, unpublished data). But while copper may be efficacious for disease control and is relatively nontoxic to human handlers, managers are cautious in using copper because of its known toxicity to the salmonid olfactory organ.

The goal of this current study was first to measure the initial impacts of copper, at hatchery treatment concentrations, on the electrical properties of olfactory receptor neurons in chum salmon fry using a traditional electrophysiological method, the EOG. We also attempted to use a novel cytophysiological approach, functional dye labelling, to visually mark active neurons. We then used these two techniques to monitor olfactory recovery over 10 days.

Materials and methods

Animals

Experiments were carried out using chum salmon obtained from the Hokkaido Fish Hatchery (Eniwa, Japan) and raised at Hokkaido University Toya Lake Station for Environmental Biology (Toya, Japan) under natural photoperiod conditions. Fish were maintained in tanks supplied with filtered spring water (40 $mg \cdot L^{-1}$ total hardness as $CaCO_3$, pH 6.1, conductivity 0.14 $mS \cdot cm^{-1}$, turbidity 0.0 nephelometric turbidity units (NTU), dissolved oxygen 9.1 $mg \cdot L^{-1}$, and temperature 9.7 °C) in a single-pass flow system. Fish were raised on standard commercial salmon pellets. The chum fry were 2–3 months in age, mean length (±standard deviation, SD) 4.9 ± 0.3 cm, and weight 0.8 ± 0.2 g.

Chemical solutions

A concentrated stock copper solution was prepared at the Hokkaido Fish Hatchery using a copper ion generator (ABLE Co. Ltd., Tokyo, Japan). Similar to the methods used by hatchery managers to prepare copper solutions for disease control, our exposure solutions were prepared by adding varying amounts of copper stock to spring water in glass aquaria (25 L). Triplicate samples collected from control and exposure tanks (four concentrations of copper) were preserved with 0.1 $mol \cdot L^{-1}$ nitric acid (1% of total volume), filtered through a 0.45 µm membrane, and then analyzed for total copper content along with quality assurance copper standards and blanks. Analyses were conduced at the Hokkaido Fish Hatchery using atomic absorption spectroscopy (Hitachi Z-6000 Ltd., Tokyo, Japan). Spring water control solutions were found to contain a background level of 0.0 $\mu g \cdot L^{-1}$ total dissolved copper (detection limit 0.05 $\mu g \cdot L^{-1}$). Exposure solutions contained 3, 8, 24, and 58 $\mu g \cdot L^{-1}$ copper.

An odorant solution containing 10 $mmol \cdot L^{-1}$ L-serine (Wako Pure Chemical Industries, Tokyo, Japan) was prepared daily in spring water from a concentrated stock of the amino acid. Hatchery outlet stream water (considered the home stream for returning, station-raised fish, with a presumably complex odorant composition) was collected in a 1 L glass jar at its discharge point into Lake Toya, refrigerated, and used daily throughout the duration of the experiment. Outlet water had the following characteristics: 75 $mg \cdot L^{-1}$ total hardness as

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$CaCO_3$, pH 6.7, conductivity 0.15 mS·cm^{-1}, turbidity 2 NTU, and dissolved oxygen 11.3 mg·L^{-1}.

Copper exposures and electrophysiological recordings

Fish were treated for 4 h in copper-treated spring water (in groups of six fish each). After the exposure period, a single fish was removed from the group for electrophysiological tests, while the remaining fish were transferred to a flow-through spring water tank for recovery (for 1, 4, 7, or 10 days). This process was repeated six times at each copper concentration (n = 6 fish per copper concentration). On each recovery day, six fish per exposure group were selected for EOG tests (n = 6 fish per recovery day). At least one control fish (copper blank exposure) was tested each day over the course of the experiment (n = 15 total).

Prior to EOG tests, fish were anaesthetized in eugenol (2-methoxy-4-(2-propenyl) phenol) at 50 µL·L^{-1} and then placed in a holder on a vibration isolation table. Oxygenated spring water containing 20 µL·L^{-1} eugenol irrigated the gills through a mouthpiece. Earlier tests performed in our laboratory indicated that similar eugenol exposures did not impair olfactory sensitivity salmon (Y. Yamamoto, Division of Environmental Science Development, Graduate School of Environmental Science, Hokkaido University, North 9 West 9, Kita-ku, Sapporo, 060-0809, Japan, unpublished data). The responsive properties of the olfactory receptor neurons were recorded by using a pair of glass microelectrodes filled with 2.5% agar–saline and bridged to a silver wire. With the aid of a stereomicroscope and micromanipulators, an odorant perfusion tube was inserted gently into the in-current passage of the nare, and the recording microelectrode was inserted through the ex-current passage and positioned above the midline of the rosette at the base of posterior-most lamella. A reference microelectrode was placed on the head, and a separate ground electrode was clipped to the tail of the fish. The differential electrical signal was amplified (500×) and filtered (100 Hz low-pass) using a direct current amplifier (A-M Systems, Carlsborg, Washington, USA). The signals were digitized at 10 samples·s^{-1} using PicoScope data acquisition software (Pico Technology Ltd., St. Neots, UK), and the signal amplitudes were measured in millivolts (mV).

After electrode placement, the olfactory rosettes were rinsed for 15 min with spring water at a steady rate of 1 mL·min^{-1}. Odorants were then pulsed for 10 s into the spring water three times, each pulse separated by 150 s. EOGs were recorded in response to L-serine (10 mmol·L^{-1}) and hatchery outlet stream water. The amplitude of the EOG response was quantified by the negative phasic displacement of the odor-evoked peak relative to the prestimulus electrical baseline.

Olfactory receptor neuron labelling using AM1-43

Fluorescent dye AM1-43 ($C_{39}H_{66}Cl_2N_4$, molecular weight = 559.5) was purchased from Biotium, Inc. (Hayward, California, USA). A concentrated stock solution of the dye (100 µmol·L^{-1}) was made in distilled water and stored in 500 µL aliquots at –20 °C.

For method development, solutions of AM1-43 dye were prepared in spring water and adjusted to pH 7.0 with diluted NaOH. Dye concentrations (1, 5, 10, and 20 µmol·L^{-1}) and dye perfusion times (15, 30, 60, and 120 s) were tested to determine appropriate parameters for later copper exposure experiments. We sought a combination of these two parameters that would allow individual cells to be distinguishable and also allow either increases or decreases in labelled cell numbers to be observed clearly. We also tested the use of a fluorescence quencher (SCAS; Biotium, Inc.) for removing background staining from the epithelial surface, but found that rinsing with clean spring water was sufficient for the purpose of this study. Based on these preliminary dye experiments, immediately following EOG testing, rosettes of fish were perfused for 30 s with AM1-43 (10 µmol·L^{-1}) and then rinsed with clean spring water for 3 min. The anterior portion of the snout containing the olfactory chamber was then excised and placed overnight in approximately 10 mL of a 1% paraformaldehyde, 2.5% glutaraldehyde fixative solution. The rosette was then extracted from the olfactory chamber and embedded in 10% agar gel. Rosettes were sectioned at 30 µm using a Vibratome DTK-2000 (Dosaka EM, Kyoto, Japan).

Labelled cells were found distributed evenly among the several lamella of the rosette. For consistency between animals and for sufficient statistical power in analyses, five midregion sections from the largest, anterior-most lamella of the rosette from each fish was arbitrarily selected for cell counting. Sections were magnified at 200× using a fluorescent microscope (Axioskop, Carl Zeiss, Gotingen, Germany) filtered at 450–490 nm. Color digital images were captured using a charge-coupled device (CCD) camera (model VB-7010, Keyence, Osaka, Japan) and imaging software (model VH-H1A5, Keyence), and individual cells were counted manually. The fluorescent images were converted to gray-scale and intensity inverted using Adobe Photoshop Elements 1.0 (Adobe Systems, Inc., San Jose, California, USA) for publication.

Labelling appeared to be nonselective for cell type (e.g., microvillar, ciliated, crypt), as determined by cell morphology (Sato and Suzuki 2001; Belanger et al. 2003; Hansen et al. 2003) and position in the epithelium (Hansen and Finger 2000; Hamdani et al. 2001; Hamdani and Døving 2002). For cells to be accepted as labelled, the entire dendrite leading to the apical surface and the cell body had to be clearly defined. All olfactory receptor cell types were included in counting.

Statistics

Statistical analyses and graphing were performed using GraphPad Prism 4.0 (San Diego, California, USA). Differences in EOG amplitudes and differences in fluorescent cell numbers were compared using one-way analysis of variance (ANOVA), followed by a Dunnett post hoc multiple comparison test.

Results

EOG reduction and recovery following 4 h copper exposures

Mean EOG amplitudes (standard error, SE) stimulated by L-serine and outlet stream water were 2.8 ± 0.2 mV and 3.2 ± 0.1 mV, respectively. Typical examples of L-serine-evoked EOGs for control (0 µg·L^{-1}) and copper-exposed (3–58 µg·L^{-1}) fish are shown (Fig. 1*a*). Exposures to copper reduced the olfactory responses of chum fry to both odorants in a con-

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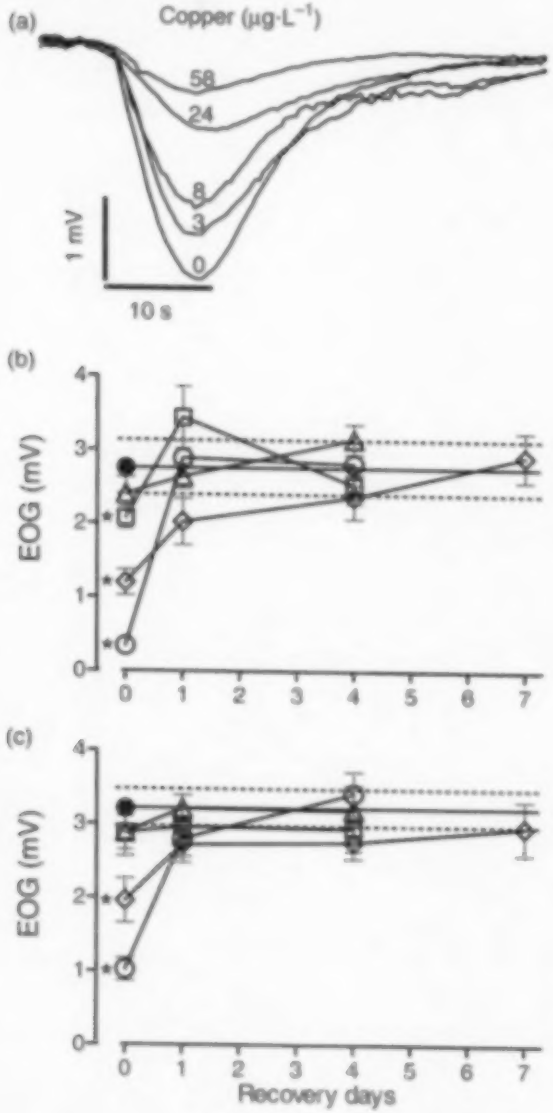
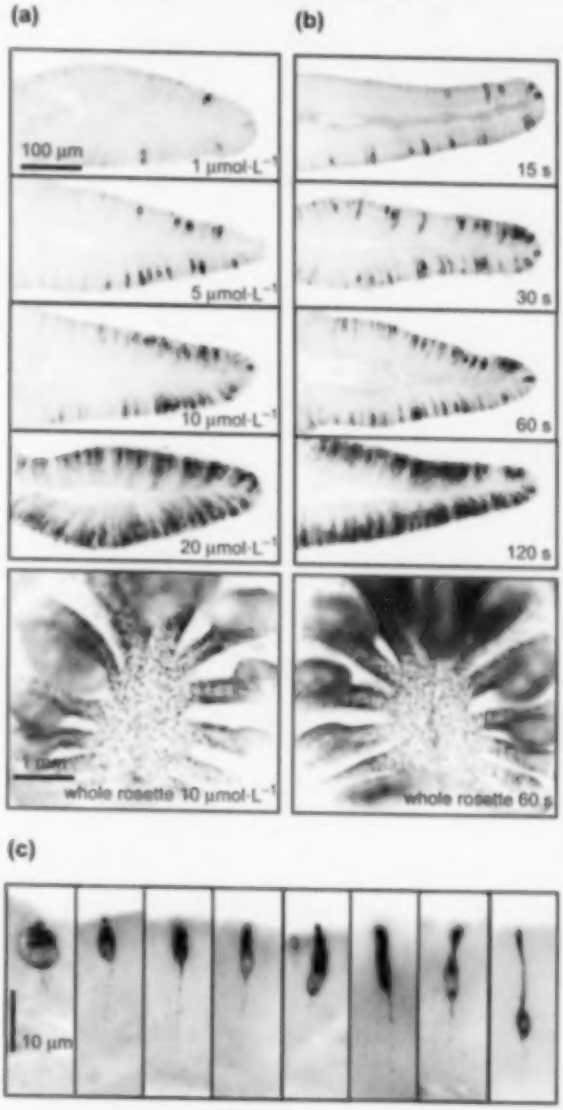
Fig. 1. (*a*) Typical examples of electro-olfactogram (EOG) recordings from chum salmon fry (*Oncorhynchus keta*) exposed to copper. Inhibition and subsequent recovery of odorant-evoked EOGs; (*b*) 10^{-7} mol·L^{-1} serine, (*c*) hatchery outlet water. Mean control EOG amplitudes are shown as horizontal solid lines extending from single solid circles; parallel dashed lines represent 95% confidence intervals. Open symbols represent copper-exposed groups: triangle, 3 μg·L^{-1}; square, 8 μg·L^{-1}; diamond, 24 μg·L^{-1}; circle, 58 μg·L^{-1}. Error bars represent ±1 standard error; asterisks indicate significant differences from the control group at $p < 0.05$.



centration-dependent manner (Figs. 1*b*, 1*c*; ANOVA, $p < 0.05$). During the recovery period over 7 days, EOG responses increased in all exposure groups and were statistically similar to the control group after 1 day and beyond (ANOVA, Dunnett test, $p > 0.05$ for all groups).

Fig. 2. (*a*) Dye concentration and (*b*) perfusion time influence the number of olfactory receptor neurons that label with AM1-43, as shown in epithelial sections (first four panels) and in whole olfactory rosettes (fifth panel). (*c*) Examples of various olfactory receptor neurons that label with AM1-43.



Fluorescent labelling of olfactory receptor neurons following copper exposures

Increasing dye concentration and dye perfusion time subsequently increased the number of olfactory receptor neurons that labelled in fish not exposed to copper. The labelled neurons were distributed evenly throughout the intact rosette, and typical examples of epithelial sections and whole rosettes revealing individually labelled neurons are shown (Figs. 2*a* and 2*b*, respectively). Various olfactory receptor neurons (cell types not specified) that internalized AM1-43 are shown (Fig. 2*c*). Labelling did not appear to be odorant-

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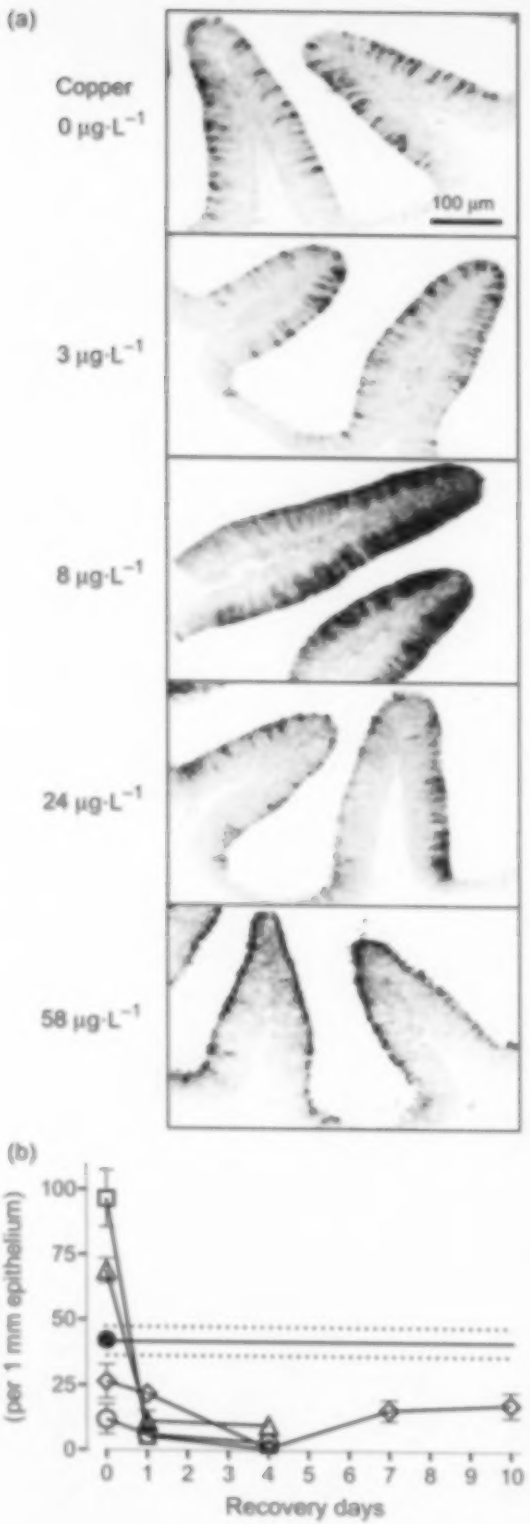

Fig. 3. (*a*) Copper exposures alter the number and pattern of olfactory receptor neurons that label with AM1-43. (*b*) The mean number of labelled cells from controls is shown as a horizontal solid line extending from a single solid circle; parallel dashed lines represent a 95% confidence interval. Open symbols represent copper-exposed groups: triangle, 3 µg·L^{-1}; square, 8 µg·L^{-1}; diamond, 24 µg·L^{-1}; circle, 58 µg·L^{-1}. Error bars represent ±1 standard error; all exposure groups were significantly different from the control group at $p < 0.05$.

dependent, as indicated by additivity tests in which varying concentrations of L-serine (10^{-3}, 10^{-4}, and 10^{-5} mol·L^{-1}), combined with AM1-43 (10 µmol·L^{-1}), increased the amplitude of the EOG, but did not alter the number of cells that labelled with dye (data not shown).

Exposures to copper altered the pattern and number of olfactory receptor neurons that labelled with AM1-43 (Figs. 3*a*, 3*b*). In the control group of fish, the mean number (±SE) of labelled cells per 1 mm of epithelial tissue, at a thickness of 30 µm, was 42.2 ± 5.5 cells. After 4 h exposures to 3 and 8 µg·L^{-1} copper, the number of cells that labelled increased significantly (68.8 ± 4.7 and 96.5 ± 10.8 cells, respectively) as compared with the control group (ANOVA, Dunnett's test, $p < 0.05$). However, at 24 and 58 µg·L^{-1} there were significant decreases in the number of cells that labelled (26.4 ± 7.1 and 11.8 ± 5.7 cells, respectively) as compared with the control group (ANOVA, Dunnett's test, $p < 0.01$). In the 24 µg·L^{-1} copper group, internalization of the dye was often confined to the apical regions of the dendrites, and some background staining of debris occurred at the surface of the epithelium. At 58 µg·L^{-1} copper, very few cell bodies or dendrites internalized AM1-43, and the surface of the epithelium showed substantial background staining.

Unlike the EOGs, fluorescent labelling of cells did not return to the level of the control group after 1 day (Fig. 3*b*). In fact, the number of cells that labelled in all copper-exposed groups declined below the control number, which continued through day four. The 24 µg·L^{-1} exposure group was monitored for 10 days, after which the mean number of labelled cells showed only a partial return to the control group number.

Discussion

The degree of copper toxicity to the olfactory system in fish is dependent on both exposure concentration and exposure time (e.g., Saucier and Astic 1995). The combination of these parameters (in addition to other parameters, such as water chemistry, acclimation of fish to the test water, etc.) will influence the degree of impact on target sites and ultimately whether the toxic effects are reversible (short-term) or irreversible (long-term). The initial targets of copper on olfactory receptor neurons are believed to be proteins associated with odorant receptors, ion channels, and intracellular transduction mechanisms (Rhenberg and Schreck 1986; Winberg et al. 1992; Bjerselius et al. 1993). Longer exposures to copper at relatively high concentrations can damage cilia and microvilli located at the cellular surface, rupture the cellular membrane, or critically impair the protein-rich mitochondria and other organelles of the cytoplasm (Moran

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et al. 1992; Hansen et al. 1999). Severe damage to olfactory receptor neurons can lead to necrosis or apoptosis, requiring the entire regeneration of receptor cells (Moran et al. 1992; Julliard et al. 1996; Hansen et al. 1999). In this current study, electrophysiological olfactory function in chum salmon fry recovered to control levels after just 1 day, indicating that the toxic effect of copper on the electrical properties of the neurons was generally reversible.

The fluorescent dye AM1-43 has previously been used as a nerve terminal probe to follow neurotransmitter activity at central or neuromuscular synapses (Henkel et al. 1996; Kay et al. 1999; Pyle et al. 1999) or to measure activity of sensory cells triggered by mechanotransduction mechanisms (Meyers et al. 2003; Gale et al. 2001). AM1-43 has two carbon chain lipophilic tails at one end and a highly hydrophilic, cationically charged head group at the other. AM1-43 can enter nerve terminals by inserting into the outer layer of the cellular membrane, then moving into the cell via clathrin-mediated endocytosis within packets containing recycled neurotransmitter molecules. It can also enter the cells via certain large, nonspecific ion channels of the sensory neurons (Meyers et al. 2003).

Here, we show that AM1-43 can also label olfactory receptor neurons in intact fish by perfusing the dye over the olfactory rosette. Labelling was not dependent on the presence of an odorant. The number of labelled cells did not increase when AM1-43 was combined with L-serine, suggesting that internalization of the dye does not occur via a pathway related to the neuron electrical generator potential. Exposures to copper dramatically changed labelling patterns of the neurons, altering AM1-43 uptake into cells in a biphasic, concentration-dependent manner by an unspecified mechanism.

Although copper can be highly toxic to the olfactory neuron, it is also an essential trace metal. Copper is required for the proper functioning of several enzymes and has been shown to play an important role in odorant binding for some receptor proteins (Wang et al. 2003). Copper is also necessary in the synthesis of certain neuropeptides within the olfactory system (Rutkoski et al. 1999) and is believed to be a neuromodulator at various receptor synapses of the olfactory bulb (Trombley and Shepherd 1996; Acuna-Castillo et al. 2000; Horning et al. 2000). Studies in both mammals and fish have shown that certain heavy metals are actively directed to the olfactory bulb via anterograde axonal transport from the epithelium (Gottofrey and Tjälve 1991; Tallkvist et al. 1998, 2002). However, to our knowledge, a specific mechanism for the active uptake of the metals by olfactory neurons at the epithelial surface has not yet been identified.

One such mechanism may be similar to that of human kidney cell lines. These cells contain human copper transporter (hCtr1) proteins at the membrane surface that assist in the uptake and regulation of cellular copper (Petris et al. 2003). This protein is continuously recycled at the plasma membrane, even in the absence of copper, by clathrin-mediated endocytosis, and the vesicles are then trafficked to endosomes within the cell. Under low copper conditions, Petris et al. (2003) found an up-regulation of hCtr1 protein expression at the plasma membrane, which in turn, would serve to elevate uptake of copper via increased endocytosis, possibly of a copper–hCtr1 complex. Higher levels of copper caused degradation of the hCtr1 protein at the plasma membrane, which in turn may have provided a protective mechanism to restrict further copper uptake. Fish olfactory receptor neurons may contain a similar metal-sequestering mechanism (Tjälve et al. 1986; Horning and Trombley 2001; Tallkvist et al. 2002). In the brown trout (*Salmo trutta*), various tissues, including the olfactory apparatus and kidney, also accumulated dissolved metal in a biphasic, concentration-dependent manner (Tjälve et al. 1986). Lower cadmium exposures resulted in higher tissue metal accumulations, whereas the pattern was reversed when the fish were exposed to higher cadmium concentrations. Further research is necessary to identify the specific mechanism for copper regulation in the olfactory receptor neurons of fish.

Under our experimental conditions, the electrophysiological responses of primary olfactory neurons in chum salmon fry recovered from short-term copper exposures after 1 day. For hatchery salmon, this brief disruption to olfaction may not be life-threatening or cause long-term harm. Fish in the wild are not afforded the protection of a hatchery, however, and even a relatively short loss of olfactory ability could impair essential survival strategies (Beyers and Farmer 2001; Mirza and Chivers 2001). Although electrical properties of the olfactory system recovered quickly in this study, unspecified cytophysiological properties of the olfactory receptor neurons remained altered up to 10 days after exposure, as suggested by sustained decreases in cells that labelled with AM1-43. The significance of this finding is not yet clear, but we believe that dyes such as AM1-43 will be useful in helping to understand mechanisms of metal regulation and toxicity in the fish olfactory system. How salmonids can acclimate, and indeed thrive, in some natural waters with relatively high copper concentrations is an important question, especially for regulators concerned about copper inputs from nonnatural sources. This will serve as the basis for future investigations.

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Spatial variability in nursery functions along a temperate estuarine gradient: role of detrital versus algal trophic pathways

Md. Shahidul Islam and Masaru Tanaka

Abstract: We aimed to identify spatial variability in estuarine trophic pathways and to elucidate their role as a nursery for juvenile fishes along Chikugo River estuary, Japan. First, we investigated distribution of larval and juvenile fishes and their diets. Using estuarine-dependent species as a model, we analyzed diets of Japanese sea bass (*Lateolabrax japonicus*) and conducted copepod distribution and gut fluorescence studies. To evaluate the nursery functions of spatially variable food webs, we used several indices: copepod biomass, feeding intensity, gut content biomass, growth rates, RNA/DNA ratio, condition factor, and starvation status of sea bass larvae and juveniles. We identified four different spatial distribution patterns of fishes: oligohaline, mesohaline, euryhaline, and polyhaline. Copepod assemblages and diets of fishes were completely different among the regions. Gut chlorophyll *a* and phaeopigment indicated that copepods in the oligohaline region depend on a detrital food web, while in the euryhaline region they depend on an algal food web. The oligohaline region had maximum turbidity, nutrients, chlorophyll pigments, and copepod biomass, and sea bass juveniles had higher feeding intensity, gut content biomass, RNA/DNA ratio, growth rate, and condition factor and lower starvation rates. This suggests that the oligohaline region, supported by a detrital food web, is an important nursery that positively influences fish growth and condition.

Résumé : Nous cherchons à identifier la variabilité spatiale des voies trophiques estuariennes et à élucider leur rôle comme nursery pour les jeunes poissons dans l'estuaire du Chikugo, Japon. Nous étudions d'abord la répartition des larves et des jeunes poissons et leur régime alimentaire. En utilisant comme modèles des espèces dépendantes de l'estuaire, nous analysons le régime du bar japonais (*Lateolabrax japonicus*), déterminons la répartition des copépodes et menons des études de fluorescence du tube digestif. Afin d'évaluer le rôle de nursery des réseaux alimentaires variables dans l'espace, nous employons plusieurs indices, soit la biomasse des copépodes, l'intensité de l'alimentation, la biomasse du contenu du tube digestif, le rapport ARN/ADN, le coefficient de condition et l'état de disette des larves et des petits. Nous distinguons quatre patrons de répartition spatiale des poissons, oligohalin, mésohalin, euryhalin et polyhalin. Les peuplements de copépodes et les diètes des poissons diffèrent complètement d'une région à l'autre. L'analyse de la chlorophylle *a* et des phéopigments dans le tube digestif indique que les copépodes de la région oligohaline dépendent du réseau trophique du détritus, alors que, dans la région euryhaline, ils dépendent du réseau trophique des algues. La région oligohaline possède le maximum de turbidité, de nutriments, de pigments chlorophylliens et de biomasse de copépodes et les jeunes bars japonais y ont le taux d'alimentation, la biomasse du contenu du tube digestif, le rapport ARN/ADN, le taux de croissance et le coefficient de condition les plus élevés et les taux de disette les plus bas. Cela laisse croire que la région oligohaline, alimentée par un réseau trophique à base de détritus, forme une importante nursery qui influence de façon positive la croissance et la condition des poissons.

[Traduit par la Rédaction]

Introduction

Estuaries are extremely dynamic and heterogenous ecotones where extreme variability in hydrographic environment occurs with tremendous influences on estuarine lives and their trophic structures. Substantial efforts have been made over years on the habitat use and feeding ecology of many estuarine-dependent species (Rakocinski et al. 1996; Elliott and Hemingway 2002; Able 2005), and the role of estuaries as nursery habitat for many commercially exploitable fish species is now well established (Beck et al. 2001). Many studies have suggested that estuarine nursery habitats contribute substantially to the recruitment success in fishes through providing higher juvenile growth and enhanced survival (Yamashita et al. 2003; Ohta 2004). Juveniles grown up in the estuarine nursery habitats contribute considerably to

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the spawning stock biomass necessary for subsequent spawning (Ohta 2004). Therefore, estuarine nursery habitats play an important role for sustaining populations of estuarine-dependent fishes. However, we still do not know much about the mechanism of estuarine nursery functions, particularly the role of estuarine food-web dynamics. Little is known about the primary producers at the base of the food web that support high secondary productivity in estuaries. Knowledge on the spatial variation in estuarine food webs is important in understanding high estuarine productivity and the mechanism of estuarine nursery functions for fishes and other estuarine-dependent species.

Estuarine food web studies have classically focused on two general paradigms: one is the simple algal food web, which is associated with the open part of the estuary and is based on in situ production of microalgae, particularly diatoms. The diatom–copepod link has been a key element in this classical, pelagic, marine food web. In contrast, the detrital food web is rather complex, associated with the riverine portion of the estuary and based on terrestrially derived detrital inputs. While the classical pelagic marine food web has been substantially studied over the last decades, much less has been known on the detrital food web, despite that the nursery areas of many estuarine fishes are formed in the riverine portion of the estuary and may be closely linked to detrital food web. Therefore, terrestrially derived detrital inputs to estuarine food webs may be important in many ecosystems (Finlay 2001; Finlay et al. 2002). However, the sources of production to estuarine food webs at a given location, their regional variability, and the role of detrital food web as a nursery for estuarine-dependent, commercially exploitable species are poorly known. This limited resolution of the resource base of estuarine food webs constrains our basic understanding of estuarine food-web ecology. Such information is crucial to understanding processes controlling the dynamics of local populations, communities, and ecosystems (Finlay et al. 2002).

The estuarine turbidity maximum (ETM) is a ubiquitous feature in many estuaries that greatly influences the heterogeneity of estuarine ecosystems. The physical condition of ETM results in accumulation and retention of high-density planktonic organisms. ETM is believed to be advantageous for plankton feeding and survival because planktonic predators encounter more prey under conditions of high turbidity (Rothschild and Osborn 1988; Roman et al. 2001; Winkler et al. 2003). Consequently, high plankton concentrations occur in areas of turbidity maxima (MacKenzie and Kiørboe 2000; Visser et al. 2001; David et al. 2005). The turbid regions of many estuaries are composed of resuspended detrital particles or allochthonous materials that are colonized by bacteria, resulting in enhanced bacterial activity (Plummer et al. 1987; Crump and Baross 1996). Owing to the abundance of particle-attached bacteria, bacterial carbon may be packaged in many particle sizes and may be available as food to the grazers (Crump and Baross 1996), which, in addition to high in situ phytoplankton and benthic microalgal concentrations, contribute to higher secondary production in ETM zones. In contrast, the concentration and influence of detrital materials is minimal in the open part of an estuary where autochthonous primary production is the sole source of food for grazers. Thus, ETM zones are characterized by considerably higher secondary production than the downstream areas (Laprise and Dodson 1994; Roman et al. 2001; Winkler et al. 2003). Several studies have shown that terrestrially derived carbon makes important contributions to the food web in the upper riverine portion of estuary, but the riverine-borne organic matter is of minimal importance to higher trophic levels in the lower, open part of estuary (Simenstad and Wissmar 1985; Bunn et al. 1989). Therefore, substantial spatial variability between the upper and lower estuary may be expected at the base of the food webs.

Previous studies have shown that ETM is advantageous for larval and juvenile fish feeding and survival because ETM regions are characterized by higher prey concentrations. Thus, larval and juvenile fish predators encounter more prey under conditions of elevated turbidity because of increased encounter rates (Rothschild and Osborn 1988). Therefore, larvae in the ETM zones experience higher feeding success, which may increase the chance of survival; subsequently, high abundance of larval and juvenile fishes occurs in areas of turbidity maxima (North and Houde 2003). ETM usually corresponds to areas just near the salt front where fresh water mixes with seawater, and therefore, ETM zones have its characteristic zooplankton communities that are different from that found in upper freshwater and lower euryhaline marine areas (Laprise and Dodson 1994; Roman et al. 2001; Winkler et al. 2003). Therefore, it could be expected that the dietary habits of the fishes in the ETM differ from that of the downstream marine assemblage, which implies that estuarine food webs may be spatially variable and may be associated with the ETM processes. Although ETM processes have been substantially studied in the major estuarine systems of the world (Sirois and Dodson 2000; Bennett et al. 2002; North and Houde 2003), the spatial variability in estuarine food webs and the resulting variation in growth and condition of fish larvae have not been sufficiently documented.

In this paper, we report empirical evidence of spatial variability in estuarine food webs occurring in the Chikugo River estuary, Ariake Bay, Japan. This study was conducted with two major objectives: (*i*) to characterize the spatial variability in estuarine trophic pathways and (*ii*) to evaluate the nursery functions of the spatially variable trophic pathways for larval and juvenile fishes. We adopted a top-down approach to study the dietary relations among the trophic levels in three regions: oligohaline upper estuary, mesohaline middle estuary, and euryhaline lower estuary. First, we studied the diets of the dominant larval and juvenile fishes. Then we investigated the source of energy of the major prey copepods (i.e., which plant source forms the base of the food web). Finally, we evaluated the nursery functions of the spatially different trophic pathways (i.e., influence of different trophic pathways on the growth and condition of fish juvenile).

Materials and methods

Study area

Ariake Bay (Fig. 1) is a semi-enclosed and well-mixed bay, with high tidal amplitudes that often exceed 6 m. This is the largest tidal wetland in Japan, with an approximate area of 1700 km^2 (Tsutsumi 2006). The bay is fed by a mixed source from the Japan Sea, the East China Sea, and the Pacific Ocean; a considerable mass of water drains into

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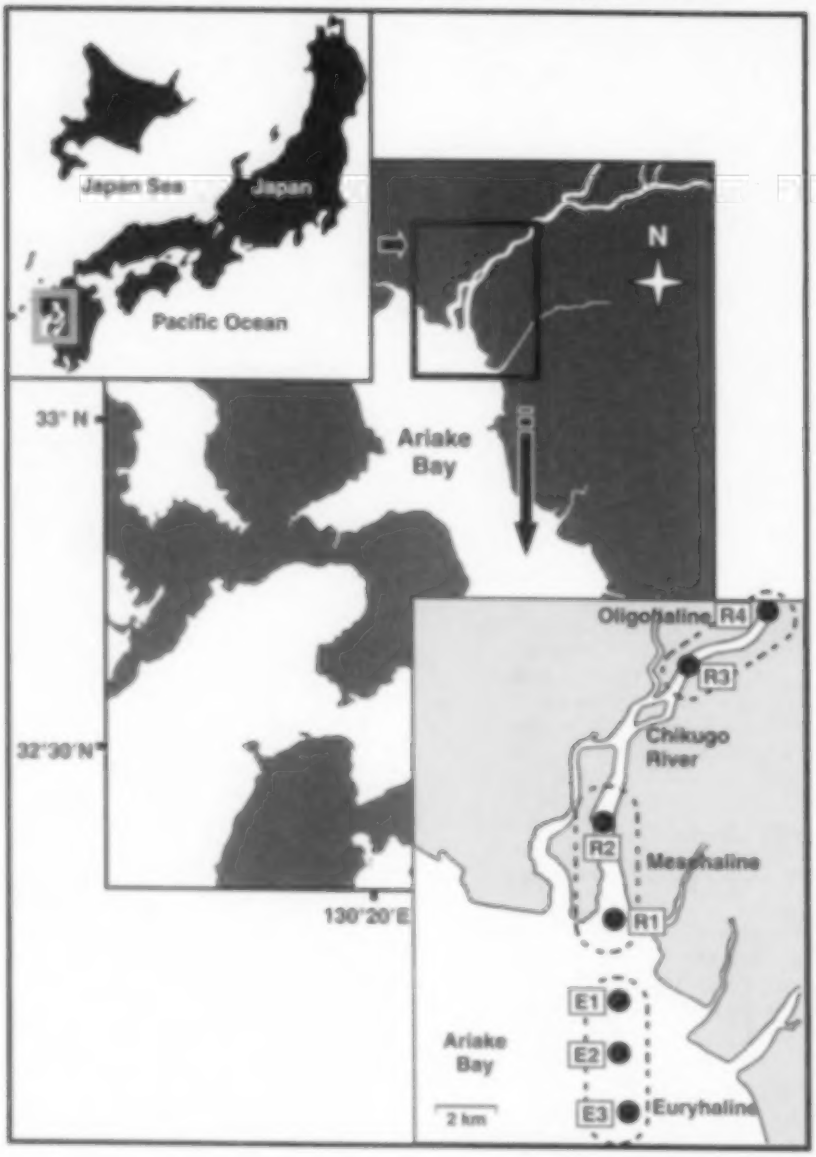
Fig. 1. Map of the Ariake Bay and the Chikugo River estuary (Japan) showing the sampling stations.



the bay from its large catchment through numerous channels. The Chikugo River is the largest river flowing into the bay at its upper part, forming a large and dynamic estuary with well-mixed water column. A nutrient-rich zone of both turbidity maxima and chlorophyll maxima occurs in the oligohaline upper part of the estuary that corresponds to the upward limit of salt intrusion. The upper estuary (oligohaline zone) is characterized by terrestrial input of dissolved and particulate organic matters and is dominated by submerged vascular plants along the riverbanks. The middle estuary (mesohaline zone) is protected from direct input of terrestrial discharge and is devoid of aquatic vegetation. The lower estuary (euryhaline zone) is an open bay with shallow tidal sand flats and is without any vegetative cover. Spatiotemporal patterns in the hydrographic environment along the estuary have not been characterized with sufficient details.

Sample collection and analysis

For sampling, a distance of about 25 km along the longitudinal axis of the Chikugo River estuary was divided into three major regions based on salinity: the oligohaline, mesohaline, and euryhaline regions. The oligohaline region started at the salt front of the upper estuary, 16 km upstream from the river mouth and ended at about 4 km downstream. The mesohaline region started at 4 km upstream from the river mouth and ended at the river mouth. The euryhaline region

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started at about 4 km downstream from the river mouth and covered a distance of about 8 km toward downstream (Fig. 1). The average salinity of the oligohaline, mesohaline, and euryhaline regions was 1.6, 12.1, and 21.2 PSU (practical salinity units), respectively. Samples were collected from two stations in the oligohaline and mesohaline regions each and from three stations in the euryhaline regions.

Field samples

Spring 2001

Larval net surveys were made during four sampling cruises on 11 and 23 March and 10 and 22 April 2001. The aim of the surveys was to investigate the ichthyoplankton assemblages, distribution patterns, and diets of dominant species along the estuary. Larval and juvenile fishes were collected by a total of 56 tows (oligohaline region: 16 tows at two stations; mesohaline region: 16 tows at two stations; and euryhaline region: 24 tows at three stations). Samples were collected by surface tows of a larva net (1.3 m mouth diameter, 5 m long, 1 mm mesh at the body, and 0.33 mm mesh at the cod end) equipped with a flow meter. At each station, the net was towed for 10 min against tidal flow between 0900 and 1200 h. Samples were immediately fixed in 10% formalin solution. In the laboratory, all fishes were sorted and identified to the lowest possible taxonomic level, and the developmental stages were characterized. For each species, total numbers were counted and standard lengths were measured to the nearest 0.1 mm. Results of fish density were expressed as the number per cubic metre of water.

Among the fishes collected, eight dominant species (*Acanthogobius hasta*, *Lateolabrax japonicus*, *Trachidermus fasciatus*, *Engraulis japonicus*, *Acanthogobius flavimanus*, *Coilia nasus*, *Sebastes inermis*, and *Neosalanx reganius*) were selected for gut content analysis. Prey organisms were counted and identified to the lowest possible taxonomic level. Feeding intensity was calculated as the total number of prey items per fish. Feeding incidence was calculated as the percentage of the number of fishes with food in relation to the total number analyzed, considering only fishes with identifiable gut contents.

Spring 2002 and 2003

Distribution patterns obtained from spring 2001 samples showed that Japanese sea bass (*Lateolabrax japonicus*) was the only species distributed in all three regions of the estuary. Therefore, this fish was used as a model estuarine-dependent species to observe between-region variability in larval growth and condition and to compare the nursery roles of the three regions. Samples were collected during March 2002 and 2003. For studying condition of juvenile sea bass, a total of eight tows were carried out in the oligohaline and mesohaline region each, and 12 tows were carried out in the euryhaline region. Several indices were used to evaluate the nursery functions among the regions: copepod biomass, feeding intensity, gut content biomass, growth, and condition of sea bass larvae and juvenile. Growth and condition were assessed using protein growth rate (G_{pi}), RNA/DNA ratio, starvation status, and condition factor derived from length–weight relationships. A total of 135 larvae and early juveniles of sea bass ranging in length from 14.8 to 26.9 mm (mean 20.8 ± 2.3 mm) and weight from 18.1 to 180.5 mg (mean 71.0 ± 26.9 mg) were used for nucleic acid analysis. Measurements of RNA, DNA, and protein contents were carried out for individually frozen fish samples, and each individual fish was used for RNA, DNA, and protein analyses. Instantaneous G_{pi} was calculated from the larval growth model proposed by Buckley (1984) based on the RNA/DNA values and temperature. The growth model was manipulated according to Robinson and Ware (1988) to calculate the critical values of the RNA/DNA ratio. The critical ratio is the theoretical ratio where there is no net protein growth (i.e., $G_{pi} = 0$) in a larval fish at a specified temperature. Details on the methods used for extraction of nucleic acids and protein and calculation of G_{pi}, critical RNA/DNA ratio, starvation rates, and condition factor have been described in our previous paper (Islam et al. 2006*a*). In addition, diets of sea bass juveniles were revisited to compare with that recorded from samples collected in 2001.

Samples for investigating copepod assemblage, distribution, and abundance were collected during March 2001–2003. During each sampling, plankton net tows were conducted in duplicate in the oligohaline and mesohaline region and in triplicate in the euryhaline region. Copepod samples were collected by oblique tows of a plankton net (45 cm mouth diameter and 0.1 mm mesh size) equipped with a flow meter. The contents of the cod end were poured into a plastic container and immediately fixed in 10% seawater formalin and transported to the laboratory. Copepods were sorted from the suspended particles and detritus under a binocular stereomicroscope. All copepods were identified to the lowest possible taxonomic level and counted; copepod density was expressed as number per cubic metre and biomass as millgrams per cubic metre of water.

Samples for copepod gut fluorescence study were collected during March and April 2003. For copepod gut fluorescence study, a copepod sample collected by the same method was poured onto a 0.25 mm sieve bag, washed, and immediately frozen in dry ice and transferred to the laboratory and subsequently frozen at –85 °C. Four dominant species (*Sinocalanus sinensis*, *Acartia omorii*, *Oithona davisae*, and *Paracalanus parvus*) based on abundance were selected for analysis, and a total of 12 replicated analyses were done at each region. Details on the methods for analyzing chlorophyll *a* (chl *a*) and phaeopigment (php) in copepods, referred to as the gut fluorescence, have been described in our previous paper (Islam et al. 2005).

Environmental parameters were studied during all cruises (2001–2003) and are presented as the mean (±SE) values. Temperature (°C), salinity (PSU), turbidity (nephelometric turbidity units, NTU), chl *a* ($\mu g \cdot L^{-1}$), and php ($\mu g \cdot L^{-1}$) were analyzed at each region during each cruise. Temperature, turbidity, and salinity were recorded on the board by an Environmental Monitoring System (YSI 650 MDS, YSI Incorporated, Ohio, USA). Details on the methods for analyzing suspended particulate matter (SPM), chl *a*, and php have been described in our previous paper (Islam et al. 2005).

Statistical analysis

Two-way analysis of variance (ANOVA) was used to examine the interannual and regional variability in hydrological parameters, copepod density, and copepod dry biomass. Regional variations in feeding intensity, gut content dry weight,

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Table 1. Two-way analysis of variance (ANOVA) summary for spatial (oligohaline, mesohaline, and euryhaline regions) and interannual (2001–2003) variations in hydrographic environmental parameters, particulate nutrients, and pigment concentrations in the Chikugo River estuary, Ariake Bay, Japan.

Parameter	Source of variation	df	*F*	*P*
Temperature (°C)	Year	2	2.04	>0.05
	Region	2	0.13	>0.05
	Year × region	4	0.19	>0.05
	Error	45	—	—
Salinity (practical salinity units)	Year	2	12.20	<0.001
	Region	2	834.61	<0.001
	Year × region	4	1.74	>0.05
	Error	45	—	—
Dissolved oxygen ($mg \cdot L^{-1}$)	Year	2	9.67	<0.001
	Region	2	1.21	>0.05
	Year × region	4	3.62	<0.05
	Error	45	—	—
Turbidity (nephelometric turbidity units)	Year	2	4.45	<0.05
	Region	2	4.86	<0.05
	Year × region	4	4.47	<0.05
	Error	45	—	—
Particulate organic carbon ($mg \cdot m^{-3}$)	Year	2	0.45	>0.05
	Region	2	21.81	<0.001
	Year × region	4	0.13	>0.05
	Error	45	—	—
Particulate organic nitrogen ($mg \cdot m^{-3}$)	Year	2	1.23	>0.05
	Region	2	37.16	<0.001
	Year × region	4	0.59	>0.05
	Error	45	—	—
Chlorophyll *a* ($\mu g \cdot L^{-1}$)	Year	2	36.85	<0.001
	Region	2	0.32	>0.05
	Year × region	4	12.37	<0.001
	Error	45	—	—
Phaeopigment ($\mu g \cdot L^{-1}$)	Year	2	2.19	>0.05
	Region	2	45.49	<0.001
	Year × region	4	1.60	>0.05
	Error	45	—	—

G_{gut}, RNA/DNA ratio, and condition factor of sea bass juveniles were evaluated by one-way analysis of covariance (ANCOVA), using length and weight as covariates. During ANOVA, homogeneity of variance was tested and data was transformed if necessary. The ANOVA and ANCOVA were followed by post hoc multiple comparisons using Tukey's test (accounts for unequal sample size) to compare the significant means ($P < 0.05$). Mann–Whitney *U* test was used to compare the amount of ambient and copepod gut chl *a* and php at each region. All analyses were done using SPSS 12.0 for Windows (SPSS Inc., Chicago, Illinois).

Results

Distribution of fish and copepods: differential trophic pathways

Temperature, dissolved oxygen, and chl *a* did not show significant regional variations ($P > 0.05$; Table 1). The lowest mean salinity was recorded in the oligohaline region, the highest in the euryhaline region, and an intermediate value in the mesohaline region (Fig. 2), with significant regional and interannual variations ($P < 0.001$). Turbidity, particulate organic carbon, particulate organic nitrogen, and php showed significant regional variation ($P < 0.05$; Table 1) with the maximum value at the oligohaline region (Fig. 2). Thus, the oligohaline region was the zone of maxima for turbidity, nutrient, and pigment.

A total of 26 242 larvae and juveniles constituting 27 species under 15 families were collected; however, only eight species contributed more than 99% of the total abundance (Table 2). *Acanthogobius hasta* was the most abundant taxon contributing 73.0% of the total catch, which was followed by *Lateolabrax japonicus* contributing 15.6% and *Trachidermus fasciatus* contributing 7.9% of the total catch. Spatial patterns of fish distribution were highly influenced by salinity. There were four different categories based on the spatial distribution: (*i*) those distributed in the oligohaline zone in the upper estuary (*Acanthogobius flavimanus*, *Coilia nasus*, and *Neosalanx reganius*); (*ii*) those distributed in the mesohaline zone at the middle of the estuary (*Acanthogobius hasta* and *Trachidermus fasciatus*); (*iii*) those distributed in the euryhaline zone at the lower estuary (*Engraulis japonicus* and *Sebastes inermis*); and (*iv*) those distributed in all three regions (*Lateolabrax japonicus*) (Fig. 3). Although *Acanthogobius hasta*

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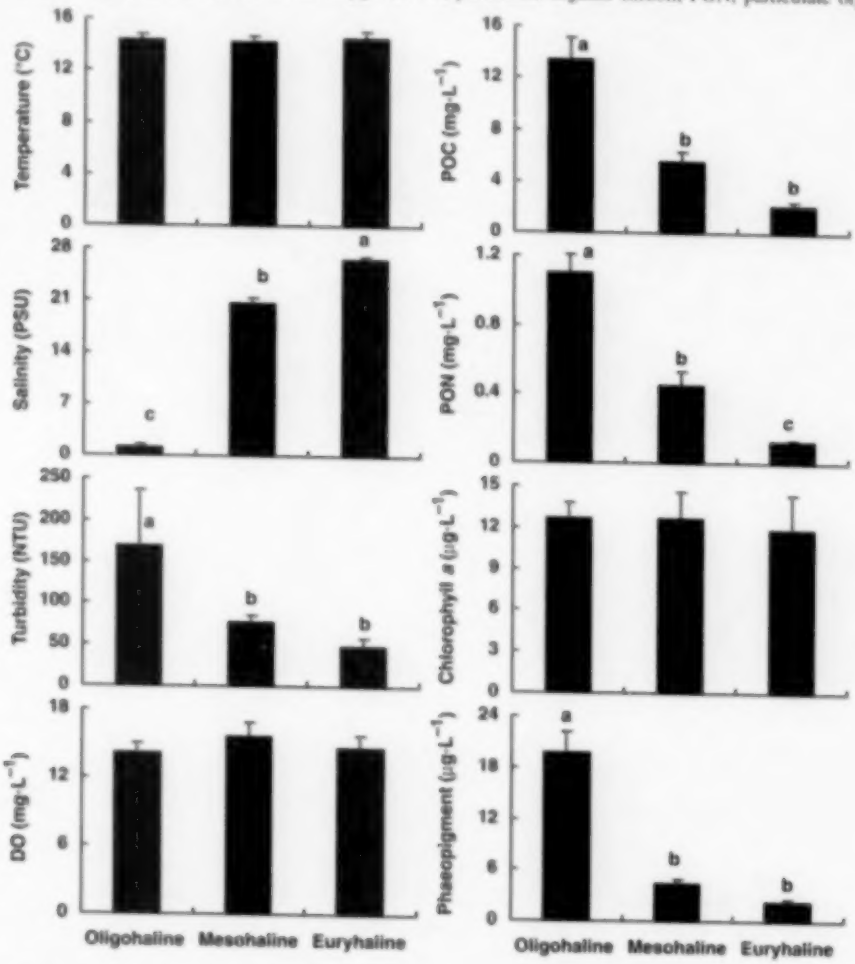
Fig. 2. Spatial variations in the hydrographic environmental parameters in the oligohaline, mesohaline, and euryhaline regions along the Chikugo River estuary. Values are mean (+ standard error) from 2001–2003 data. The letters assigned to each mean value indicate the significance of difference; mean values having different letters are significantly different ($P < 0.01$) from each other. PSU, practical salinity units; NTU, nephelometric turbidity units; DO, dissolved oxygen; POC, particulate organic carbon; PON, particulate organic nitrogen.



and *Trachidermus fasciatus* densities were higher in the mesohaline region, there was no significant variation ($P > 0.05$). Similarly, *Engraulis japonicus* and *Sebastes inermis* showed no significant difference ($P > 0.05$) despite their higher densities in the euryhaline region. This might be due to the small number of samples and high variance. *Lateolabrax japonicus* was the only species distributed in all regions with no significant regional variation in density (Fig. 3).

Gut contents of the fishes in oligohaline and mesohaline regions were highly dominated by *Sinocalanus sinensis* (Fig. 4), which contributed 35.1%, 65.9%, 87.7%, 99.3%, and as high as 100% of the diet of *Acanthogobius flavimanus*, *Trachidermus fasciatus*, *Neosalanx reganius*, *Coilia nasus*, and *Acanthogobius hasta*, respectively. In contrast, fish species in the euryhaline region showed multispecies dietary habits and preyed on several copepod species (Fig. 4), such as *Acartia omorii*, *Paracalanus parvus*, *Oithona davisae*, *Pseudodiaptomus marinus*, *Tortanus derjugini*, and unidentified copepod nauplii. *Acartia omorii* contributed 58.0% of the diet of *Engraulis japonicus*, and *Oithona davisae* contributed 64.2% of the diet of *Sebastes inermis*. *Lateolabrax japonicus* was distributed in all regions but differed in dietary habits among regions (Fig. 5). *Sinocalanus sinensis* was the major prey item in the oligohaline and mesohaline regions, while *Acartia omorii*, *Paracalanus parvus*, and *Tortanus derjugini* dominated the diet in the euryhaline region. Diets of *Lateolabrax japonicus* larvae and juveniles as well as the ambient copepod composition were almost consistent over the 3 years studied. Similar to the diets of fishes, ambient copepod composition showed clear regional variations. In the oligohaline zone, *Sinocalanus sinensis* was overwhelmingly dominant, while *Oithona davisae* dominated in the mesohaline and the euryhaline zones (Fig. 5).

Diets of dominant copepod species (determined by gut fluorescence analysis) in relation to ambient pigments (Fig. 6) showed that the ambient chl *a* concentrations did not differ significantly among regions ($P > 0.05$), while php showed significant regional variation ($P < 0.001$). In the oligohaline

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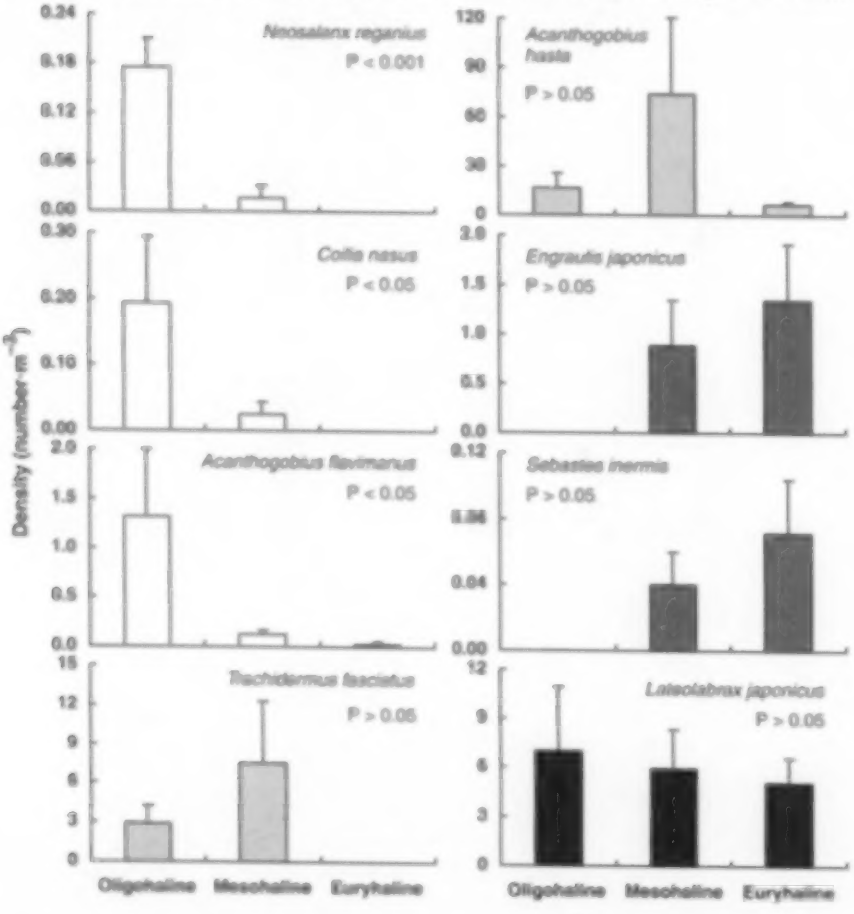
Table 2. Size range (standard length (SL), mm), mean (±SD) size (mm), catch composition (%), and the developmental stages of the most dominant fish species collected during four sampling cruises in March and April 2001.

Species	SL	Mean (±SD) size	% total catch	Stage
Acanthogobius hasta	3.9–15.5	9.0±3.9	73.0	Yolk sack juvenile
Lateolabrax japonicus	13.5–35.0	23.6±6.7	15.6	Juvenile
Trachidermus fasciatus	6.9–24.8	14.1±5.3	7.9	Preflexion juvenile
Engraulis japonicus	12.3–30.4	21.0±5.6	1.5	Flexion juvenile
Acanthogobius flavimanus	11.7–16.6	13.5±1.9	0.93	Juvenile
Coilia nasus	70.5–139.0	102.3±13.7	0.17	Young of the year
Sebastes inermis	10.8–25.6	16.6±3.6	0.12	Postflexion juvenile
Neosalanx reganius	39.7–60.7	49.2±5.7	0.14	Young of the year

Fig. 3. Density (number·m^{-3}) of dominant fish species in four different distribution patterns along the Chikugo River estuary during March and April 2001: oligohaline (distributed mostly in the low saline upper estuary; open bars), mesohaline (distributed mostly in the medium saline middle estuary; light grey bars), euryhaline (distributed mostly in the high saline lower estuary; dark grey bars) and polyhaline (distributed throughout the estuary; solid bars). Data from all stations at each zone were pooled and values are mean (+ standard error) from four sampling cruises on 11 and 23 March and 10 and 22 April 2001. *P* values are the results of analysis of variance.



region, php was significantly higher than chl *a* in ambient water (Mann–Whitney *U* test; $P < 0.05$) and in the guts of *Sinocalanus sinensis* (Mann–Whitney *U* test; $P < 0.01$). A contrasting scenario was observed in the euryhaline region, where chl *a* was significantly higher than php in ambient water (Mann–Whitney *U* test; $P < 0.01$) and in the guts of all three copepod species (Mann–Whitney *U* test; $P < 0.001$). In the mesohaline region, chl *a* and php concentrations were not significantly different in ambient water or in the guts of copepods, except in *Paracalanus parvus* (Fig. 6). Thus,

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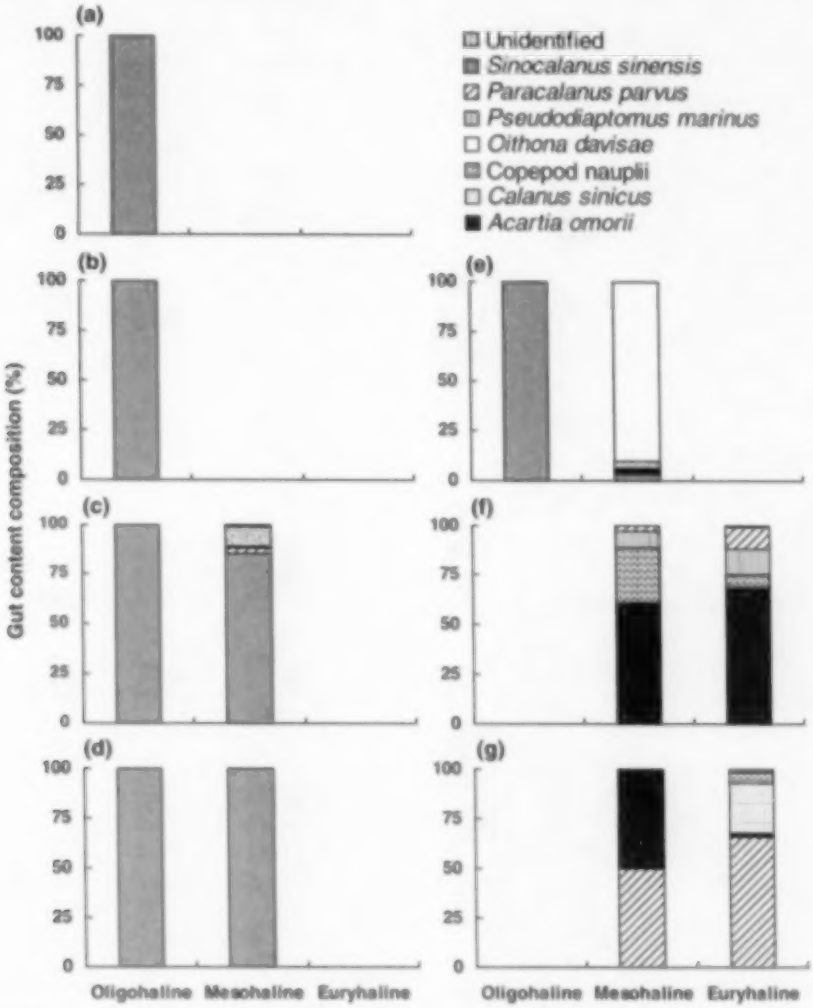
Fig. 4. Numerical composition of prey items in the guts of dominant fishes, showing contrasting dietary habits in three different regions along the estuary. Data from samples collected on 11 and 23 March and 10 and 22 April 2001. (*a*) *Neosalanx reganius*, (*b*) *Coilia nasus*, (*c*) *Acanthogobius flavimanus*, (*d*) *Trachidermus fasciatus*, (*e*) *Acanthogobius hasta*, (*f*) *Engraulis japonicus*, (*g*) *Sebastes inermis*.



copepods in the oligohaline region survive on a php-based diet (i.e., detrital diet) and those in the euryhaline region on chl *a* based diet (i.e., algal diet). Therefore, two contrasting trophic pathways exist along the spatial gradient in the Chikugo River estuary: (*i*) a detrital pathway in the oligohaline region, where fish larvae and juveniles feed on *Sinocalanus sinensis*, which survives on a detrital source and (*ii*) an algal pathway in the euryhaline region, where fish larvae and juveniles feed on *Acartia omorii*, *Paracalanus parvus*, and *Oithona davisae*, which survive on an algal source.

Nursery functions of spatially different trophic pathways

For oligohaline and mesohaline fishes, feeding intensity and feeding incidence were generally higher in the oligohaline zone than in the mesohaline zone (Table 3). In contrast, in fishes distributed in the mesohaline and the euryhaline zones, feeding intensity and incidence were generally higher in the euryhaline zone than in the mesohaline zone (Table 3). Regional variations in feeding intensity and gut content dry weight of the sea bass larvae and juveniles were significant (Table 4) and higher in the oligohaline and mesohaline regions than in the euryhaline region (Fig. 7). Analysis of covariance showed that the regional variations of feeding intensity and gut content dry weight were not confounded by the size of the sea bass larvae and juveniles (Table 4). Although copepod density did not exhibit significant regional variability, copepod dry biomass showed significant regional variation (Table 5), with the highest value at the oligohaline region (Fig. 8).

All growth and condition indices (RNA/DNA ratio, condition factor, G_{pa}) were significantly higher in the oligohaline region than in the euryhaline region (Fig. 9). In contrast,

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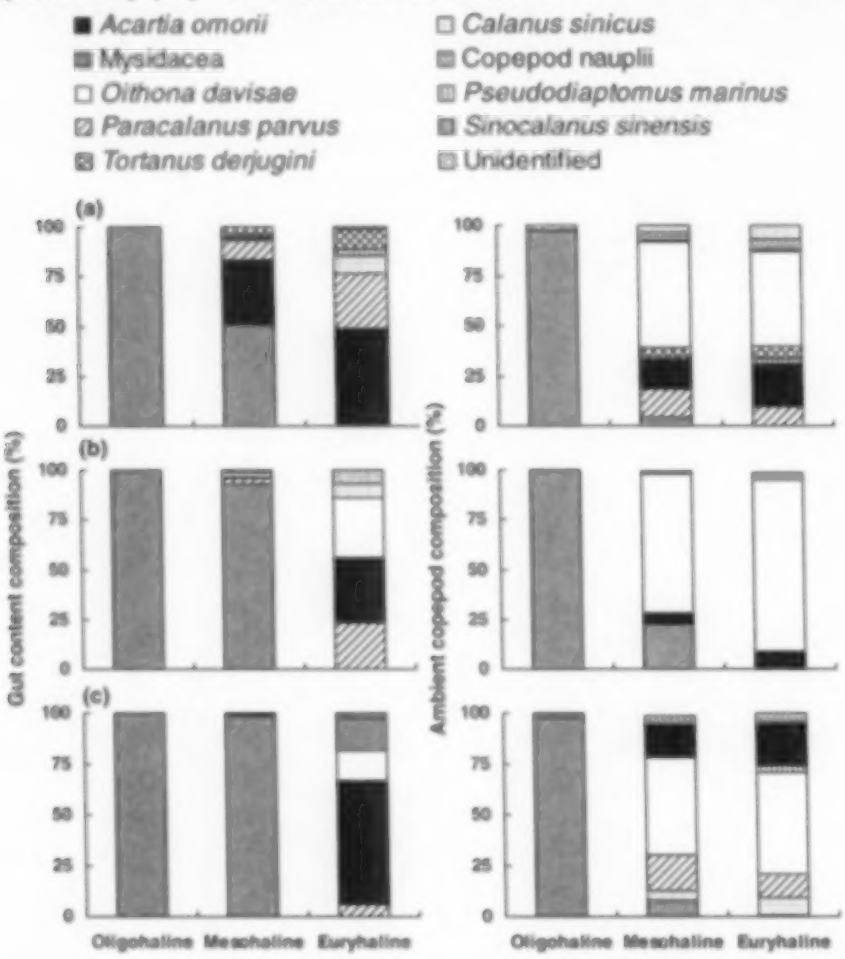
Fig. 5. Numerical composition of the gut contents of the Japanese sea bass, *Lateolabrax japonicus*, larvae and juveniles in relation to ambient copepod composition during spring of (*a*) 2001, (*b*) 2002, and (*c*) 2003.



starvation rates were significantly higher ($P < 0.001$) in the euryhaline region than in the other two regions (Fig. 9), and no fish was starving in the oligohaline region. Mean starvation rates were 0%, 5.0%, and 27.0% in the oligohaline, mesohaline and euryhaline regions, respectively. The regional variability in the RNA/DNA ratio, G_{gr}, and condition factor was not confounded by larval length, weight, or by their interactions (ANCOVA; Table 4). Clearly, fish distributed in the oligohaline region were in a better condition than those distributed in the euryhaline region.

Discussion

Distribution and diets of fishes and copepods: differential trophic pathways

Life histories of the fishes in the Chikugo River estuary are not well documented. *Acanthogobius hasta*, *Trachidermus fasciatus*, and *Acanthogobius flavimanus* use the tidal flat of the euryhaline lower estuary for spawning, and the larvae move upstream and reside the oligohaline areas of the upper estuary throughout their life (Takita and Chikamoto 1994; Kuno and Takita 1997; Onikura et al. 1999). *Coilia nasus* and *Neosalanx reganius* are residents in the fresh- to low-salinity areas of the upper estuary (Matsui et al. 1987; Takita et al. 1988; Takita 1996). Therefore, these fishes utilize the oligohaline region during most parts of their lives, and the oligohaline upper estuary is an important habitat not only for the larvae and juveniles but also for adults of these fishes. In contrast, *Engraulis japonicus* and *Sebastes inermis* are euryhaline species restricted to the open part of the bay. They are common members in the coastal pelagic fish communities along the coast of Japan (Nagasawa et al. 2000; Yukio 2000). These species are reported as obligatory resident in the offshore, high-salinity regions. The Japanese sea bass in the Chikugo River estuary shows facultative amphidromous life history; spawning occurs in the open bay and the larvae and juveniles are first dispersed into the shallow surf zone and a substantial fraction of newly transformed juveniles immigrate to the oligohaline regions above the salt front while the other fraction remain in coastal polyhaline estuarine habitats (Ohta et al. 1997). Therefore, most of the dominant fish species are dependent on the oligohaline re-

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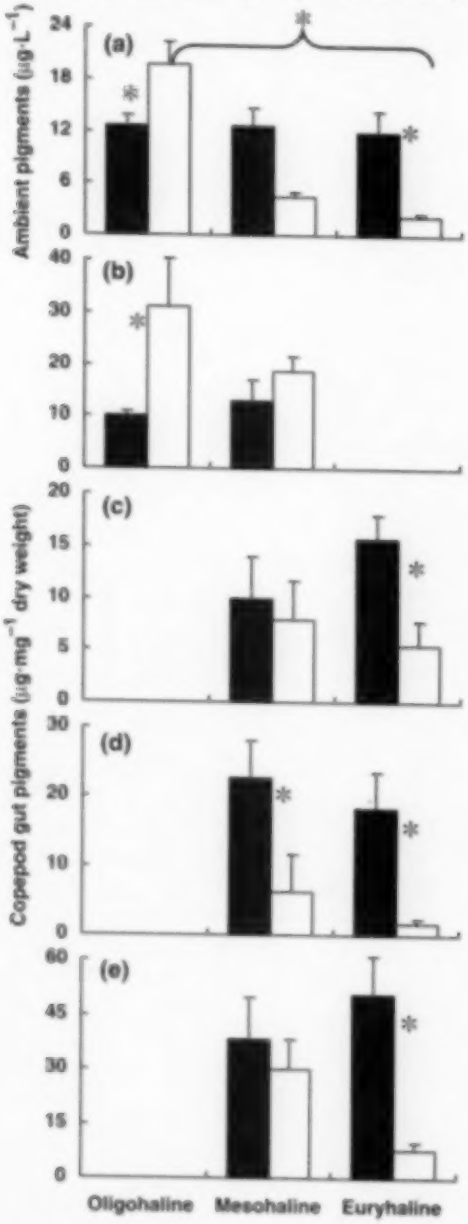
Fig. 6. Spatial patterns in gut pigment concentrations of four dominant species in relation to ambient pigment concentrations in the oligohaline, mesohaline, and euryhaline regions along the Chikugo River estuary. Values are mean + standard error obtained from samples collected during March–May 2003; (*a*) ambient chlorophyll and phaeopigment, (*b*) *Sinocalanus sinensis*, (*c*) *Acartia omorii*, (*d*) *Paracalanus parvus*, (*e*) *Oithona davisae*. Solid and open bars represent chlorophyll *a* and phaeopigment, respectively. The asterisk accompanied by the parenthesis on the topmost plot indicates significant difference in phaeopigment concentrations among oligohaline, mesohaline, and euryhaline regions (analysis of variance; $P < 0.05$); the other asterisks indicate significant difference between chlorophyll *a* and phaeopigment (Mann–Whitney *U* test; $P < 0.05$).



gion throughout their whole lives or at least during some parts of their lives. In contrast, several fish species are restricted to the euryhaline region of the lower estuary. Thus, fish assemblages showed considerable spatial partitioning between the upper and the lower estuary.

Spatial partitioning between the upper and lower estuaries occurred in copepod assemblages also. We identified two distinct copepod assemblages along the estuary: an oligohaline assemblage associated with the ETM zone in the upper estuary and a euryhaline assemblage in the lower estuary. *Sinocalanus sinensis* was the dominant species in the oligohaline region and was associated with the turbidity maximum. In contrast, *Oithona davisae*, *Paracalanus parvus*, and *Acartia omorii* were dominant species in the euryhaline region. Spatial partitioning in fish and copepod assemblages resulted in differential diets of fishes between the upper and lower estuaries. *Sinocalanus sinensis* was the single most important prey item for fishes in the oligohaline and mesohaline regions, while several other copepods, such as *Acartia omorii* and *Paracalanus parvus*, dominated the diets of fishes in the euryhaline region.

The distribution pattern of chl *a* and php in the present study suggested that most of the pigments in the ETM areas were associated with detrital particles, which contributed to high turbidity in ETM zones (Poulet 1973, 1976; Gasparini et al. 1999). Mann–Whitney *U* test revealed that php accounted for the majority of the total pigment in the upper estuary, while chl *a* accounted for the majority of the pigments in the lower estuary, indicating a clear regional variability in the contribution of two different pigment types. Significantly higher gut php concentrations in the upper estuary indicated that copepod gut content in this region was composed of detritus. In contrast, chl *a* was spatially stable, indicating that the observed regional variability in gut pigment was due to php. Significantly higher gut php concentrations than chl *a* in the upper estuary indicated that copepods in this region strongly depend on a php-based diet (i.e., detrital diet); a contrasting scenario in the lower estuary indicated that copepods in this region depend on a diet based on chl *a* (i.e., an algal diet).

Therefore, we have two scenarios in the spatial variability in estuarine trophic pathway. In the first scenario, fishes in the oligohaline region make use of a rich prey resource — an oligohaline copepod assemblage, mainly dominated by a calanoid copepod, *Sinocalanus sinensis*, which is associated with the turbidity maximum and receives energy from a detrital source. Therefore, a detrital trophic pathway exists in the ETM zone of the oligohaline upper estuary. In the second scenario, the euryhaline fishes depend on a euryhaline copepod assemblage, dominated by *Acartia omorii*, *Paracalanus parvus*, *Oithona davisae*, and some other common coastal copepods, and these copepods receive energy from an algal source. Therefore, an algal trophic pathway exists in the euryhaline lower estuary.

Spatial variations in zooplankton assemblage have been reported by many authors (Bousfield et al. 1975; Laprise and Dodson 1994; Winkler et al. 2003). These authors have reported a longitudinal succession of two well-defined assemblages of zooplankton along the St. Lawrence estuary. A true estuarine assemblage composed of *Eurytemora affinis*, *Neomysis americana*, and *Mysis stenolepis* occurred in salinities

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Table 3. Feeding incidence (percent total number of fish with identifiable gut content), feeding intensity (number of prey·fish^{-1}), number of fish analyzed, and the size range (standard length (SL), mm) of different fishes collected during four sampling cruises in March and April 2001.

Fish species	Parameter	Oligohaline	Mesohaline	Euryhaline
Acanthogobius flavimanus	No. of fish examined	12	10	—
	Size range	15.4–17.0	14.1–16.4	—
	Feeding incidence	91.7	62.5	—
	Feeding intensity	4.8	2.7	—
Acanthogobius hasta	No. of fish examined	22	20	—
	Size range	11.7–15.6	13.0–14.1	—
	Feeding incidence	72.7	0	—
	Feeding intensity	2.2	0	—
Coilia nasus	No. of fish examined	16	3	—
	Size range	104.4–121.0	102.0–114.5	—
	Feeding incidence	100	100	—
	Feeding intensity	123.4	73.3	—
Neosalanx reganius	No. of fish examined	18	3	—
	Size range	40.5–56.6	45.2–48.4	—
	Feeding incidence	100	100	—
	Feeding intensity	16.7	6	—
Trachidermus fasciatus	No. of fish examined	38	23	—
	Size range	14.6–25.0	11.2–24.2	—
	Feeding incidence	97.2	59.2	—
	Feeding intensity	39.3	5.1	—
Engraulis japonicus	No. of fish examined	—	23	40
	Size range	—	16.8–29.5	21.8–32.0
	Feeding incidence	—	57.5	77.8
	Feeding intensity	—	1.7	3.3
Sebastes inermis	No. of fish examined	—	5	19
	Size range	—	11.8–17.2	13.5–20.7
	Feeding incidence	—	37.5	56.2
	Feeding intensity	—	1	2.1

Table 4. Two-way analysis of variance (ANOVA) summary for spatial (oligohaline, mesohaline, and euryhaline regions) and interannual (2001–2003) variations in copepod density and copepod dry biomass in the Chikugo River estuary, Ariake Bay, Japan.

Parameter	Source of variation	df	F	P
Copepod density (no.·m^{-3})	Year	2	17.48	<0.001
	Region	2	0.63	>0.05
	Year × region	4	6.91	<0.01
	Error	24	—	—
Copepod dry biomass (mg·m^{-3})	Year	2	7.21	<0.01
	Region	2	4.86	<0.05
	Year × region	4	5.17	<0.01
	Error	24	—	—

of 0.5 to 5, which correspond to the ETM; this assemblage was reported as the most abundant of the estuary. At higher salinities, the estuarine community was replaced by a euryhaline marine assemblage composed of *Calanus* spp. and other commonly occurring coastal marine zooplankton. Peak abundance of *Eurytemora affinis* was reported within the ETM region in the Columbia River estuary (Simenstad et al. 1994), the San Francisco Bay estuary (Kimmerer et al. 1998), the Gironde estuary (David et al. 2005), and in the Chesapeake Bay turbidity maximum (Roman et al. 2001).

Therefore, the ETM region is an important nursery for juvenile fishes. ETM influences on the distribution and feeding of estuarine fishes have been documented for major estuarine systems. Roman et al. (2001) and North and Houde (2003) reported that the ETM region in the upper Chesapeake Bay is an important nursery area for striped bass (*Morone saxatilis*) and white perch (*Morone americana*), where larvae are retained in a region of elevated concentrations of *Eurytemora affinis* and *Bosmina longirostris*, the major prey items of striped bass and white perch larvae. Similarly, Winkler et al. (2003) reported that the ETM of the St. Lawrence estuary is an important nursery for juveniles of Atlantic tomcod (*Microgadus tomcod*) and rainbow smelt (*Osmerus mordax*). High concentrations of zooplankton

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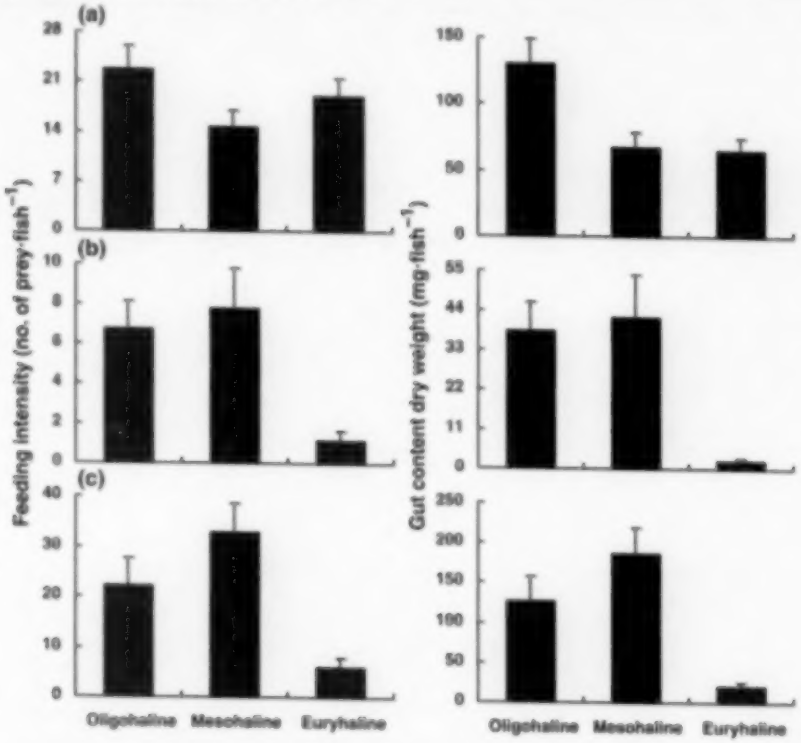
Fig. 7. Quantitative feeding estimates (feeding intensity and gut content dry weight) of the Japanese sea bass (*Lateolabrax japonicus*) larvae and juveniles in the oligohaline, mesohaline, and euryhaline regions along the Chikugo River estuary. Data are mean + standard error from samples in spring of (*a*) 2001, (*b*) 2002, and (*c*) 2003.



occur in the ETM zones because zooplankton are retained within the ETM through passive accumulation (Roman et al. 2001), and such high prey concentrations result in enhanced encounter rate by fish larvae, promoting larval feeding success (Dodson et al. 1989; Sirois and Dodson 2000). Sirois and Dodson (2000) reported that prey concentration was the most important small-scale factor affecting feeding success of white perch in the Chesapeake Bay turbidity maximum and that growth of larval rainbow smelt was enhanced at high turbidities and high prey concentrations.

Feeding ecology and food-mediated habitat shift by larval and juvenile Japanese sea bass in the Chikugo River estuary were described in our previous studies (Islam et al. 2006*a*, 2006*b*). The role of prey size and concentrations in larval feeding, associated habitat shift, and the underlying hypothesis were discussed. In the presence of large numbers of first-feeding larvae and early juveniles, greater proportions of smaller-sized prey and lower prey biomass in the downstream areas may cause density-dependent feeding, resulting in lower feeding success, which is eventually translated into poor growth larvae and juveniles (Islam et al. 2006*a*, 2006*b*) and increased susceptibility to the other sources of mortality. However, achieving faster growth is an important early survival strategy in many marine fishes. In order to achieve a high growth rate, it is critical for larvae and juveniles to consume sufficient quantities of appropriate food organisms. Therefore, ascending movement during early life stages of the Japanese sea bass could be driven by search for rich prey resources in the ETM zone. In another study (Islam et al. 2006*c*) on the distribution and diets over a long-term period (8 years), we have demonstrated that sea bass larvae and juveniles ascend to the ETM of the upper Chikugo River estuary every year, indicating that the ETM is an important and essential nursery for the fish. Therefore, sea bass larvae perform strategic ascending movement from the Ariake Bay to the upper Chikugo River estuary to reach the ETM nursery habitat.

Although species assemblages along estuarine gradients are determined by many factors, salinity appears to be the most important one that acts as a barrier and results in partitioning between oligohaline and euryhaline assemblages (Laprise and Dodson 1994). Transport to the estuarine nursery grounds and retention of fish larvae and juveniles are determined by several biological as well as physical factors. Although the exact mechanism of the ascending movement of sea bass is not comprehensively understood, Matsumiya et al. (1982, 1985) reported that bass larvae and juveniles make use of selective tidal stream transport to reach the nursery habitat. Similar mechanisms have been reported in Atlantic tomcod and rainbow smelt larvae and juveniles in the St. Lawrence estuary (Laprise and Dodson 1989; Dauvin and Dodson 1990; Winkler et al. 2003). Once the larvae and juveniles reach the nursery habitat, ETM provides the physical condition for retention in the desired location; the same mechanism is involved in the retention of copepods in the ETM. Therefore, physical conditions of the ETM result in

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Table 5. One-way analysis of covariance (ANCOVA) summary for the regional variability in feeding, growth, and condition parameters of the Japanese sea bass (*Lateolabrax japonicus*) larvae and juveniles, as well as the effects of length and weight on the spatial variations.

Dependent variable	Source of variation	df	*F*	*P*
Feeding intensity (no. prey·fish^{-1})	Main effect	3	14.80	<0.001
	Covariate; fish length	1	1.06	>0.05
	Error	390	—	—
Gut content dry weight (mg·fish^{-1})	Main effect	3	7.37	<0.01
	Covariate; fish length	1	2.50	>0.05
	Error	390	—	—
RNA/DNA ratio	Main effect	5	6.78	<0.001
	Covariate; fish length	1	1.706	>0.05
	Covariate; fish weight	1	0.213	>0.05
	Error	129	—	—
Protein growth rate (G_p; %·day^{-1})	Main effect	5	7.32	<0.001
	Covariate; fish length	1	1.735	>0.05
	Covariate; fish weight	1	0.584	>0.05
	Error	129	—	—
Condition factor	Main effect	4	79.07	<0.001
	Covariate; fish length	1	152.71	>0.05
	Covariate; fish weight	1	257.6	>0.05
	Error	129	—	—
Empty stomach (%)	Regional	2	10.02	<0.05
Starvation rate (%)	Regional	2	10.66	<0.01

high concentrations of larval and juvenile fishes as well as their prey copepods. The ETM is, in turn, influenced by other physical forcing, such the freshwater flow, which determined the location and extent of the ETM as well as its biological structure through alteration of the entrapment phenomena (Jassby et al. 1995). North and Houde (2003) proposed that variations in early life-stage retention, larval survival, and juvenile recruitment may be related to annual differences in freshwater flow that alter the physical and biological characteristics of the ETM region. Therefore, where biological conditions are structured by physical factors, the ETM region can be an important nursery area for larval and juvenile fishes and could enhance recruitment potential.

The trophic processes operating in the estuarine transition zones in relation to the coastal marine ecosystems are not well understood. Spatial variability in the estuarine food web has been reported by Deegan and Garritt (1997), Simenstad (1997), and Melville and Connolly (2005), among others. Most of the previous efforts employed stable isotope techniques to identify the source of isotopic carbon and nitrogen and their flow to the higher trophic levels, and most authors focused on the contribution of allochthonous versus autochthonous source, which has resulted in considerable controversy. Although the general paradigm is that the open estuary is little influenced by the transported organic matter and based mainly on in situ source, Melville and Connolly (2005) showed that the food webs supporting fish in Moreton Bay are based on transported organic matter, not in situ microalgae. Similar results were reported in some early studies (Incze et al. 1982; Simenstad and Wissmar 1985; Bunn et al. 1989) that suggested that riverine organic matters are of minimal importance to higher trophic levels in estuaries. However, the fact that riverine inputs and detrital food web may play important roles as nursery for estuarine-dependent fishes that spend their early life in oligohaline estuaries has not been sufficiently addressed in most of the major estuarine systems of the world. Irrespective of the variation in quantity, quality, and timing of organic matter contributing to the food web, Simenstad and colleagues showed evidence that certain species and life-history stages of juvenile salmon focus their foraging in estuaries on certain types of prey, which may be an important factor determining estuarine residence time and growth (Simenstad 1997). Such life-history patterns may have implications on their dependence on a particular food web and on their recruitment variability. A comprehensive discussion on the importance of detrital food web in salmonid ecosystem can be found in Simenstad (1997). Our observations suggest that the detrital food web provides important supports not only to the estuarine residents but also to the anadromous fishes that utilize the upper estuary as nursery habitat. Our observations are in close agreement with Simenstad and colleagues who showed the importance of a detrital food web in Pacific salmon ecosystem.

Nursery functions of detrital vs. algal trophic pathways

Between-region variations in quantitative feeding estimates (feeding intensity, feeding incidence, and gut content dry biomass) indicate the importance of the oligohaline region as the nursery for larval and juvenile fishes. Higher copepod dry biomass in the oligohaline upper estuary means higher amounts of food for fishes that resulted in higher feeding success and feeding rates and eventually higher growth. This was reflected in the growth and condition indices measured. Mean RNA/DNA values were >2.0 in all regions, which indicates that fish were growing in all regions. However, growth rates were higher in the oligohaline and mesohaline regions than in the euryhaline regions, as evident from the higher RNA/DNA values as well as higher protein growth

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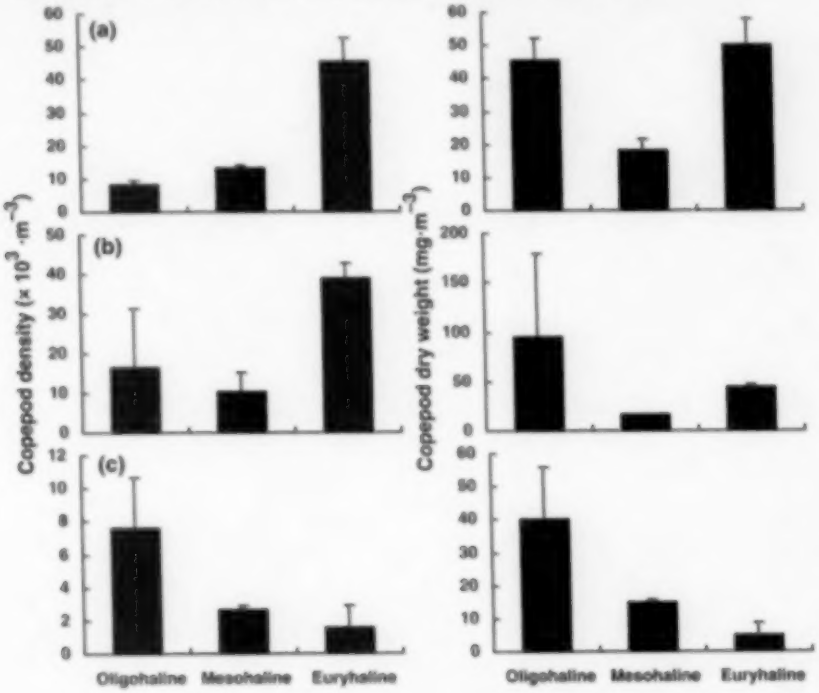
Fig. 8. Copepod density and copepod dry biomass in the oligohaline, mesohaline, and euryhaline regions along the Chikugo River estuary. Data are mean + standard error from samples in spring of (*a*) 2001, (*b*) 2002, and (*c*) 2003.



rates. Moreover, the higher condition factor clearly indicates that at a given length, the fish in the oligohaline region had higher bulk than those in the euryhaline region. Thus, the condition indices and growth rates of larval and juvenile sea bass showed that fish in the oligohaline region were in better condition than those in the euryhaline region. It may be argued that the observed differences in the growth and condition of fish larvae and juveniles are due to the fact that the larvae available in the oligohaline region are bigger in size and, therefore, stronger and already in better nutritional condition than those in the euryhaline region. To resolve the effects of fish size, we used ANCOVA, which showed that the spatial variations in the RNA/DNA values were not confounded by either larval length or weight. This pattern is consistent with previous reports (Lough et al. 1996; Chicharo et al. 1998*a*, 1998*b*) that showed no consistent relationship of condition with larval size in the field. Therefore, larvae having the same length produced variable values of RNA/DNA ratio, suggesting that the variations in larval condition were not related to fish size, and therefore, other factors were responsible for the difference in fish condition within the same size groups.

Better condition of larvae was recorded in areas where a higher copepod dry biomass, higher feeding success, and higher feeding rates were also recorded, indicating that condition was related to higher prey biomass. Several authors have suggested that changes in condition of larval and juvenile fishes may be brought about by particular prey types. Martin et al. (1985), for example, reported that the condition of striped bass in the Potomac River estuary was highly influenced by cladoceran *Bosmina* abundance; they reported that the lowest level of starvation and better condition of the fish was associated with the highest abundance of this prey species. Canino et al. (1991) studied the feeding and condition of walleye pollock (*Theragra chalcogramma*) in the Shelikof Straight of the Gulf of Alaska and found that the distribution and condition of the fish was strongly associated with the concentration of copepod prey nauplii. They reported reduced feeding intensity and RNA/DNA ratio in areas of lower nauplier concentration than in areas of higher nauplier concentration. In addition to the influence of the higher prey concentration, we also assume a causal relationship of the larval growth and condition with qualitative aspect of the diet (i.e., the spatial variations in fish condition in the present study may also be related to food quality, particularly to *Sinocalanus sinensis*, which was abundant in the upper region). Moreover, the RNA/DNA ratio is an indicator of recent feeding and growth condition. Analysis of stomach contents clearly showed that the recent feeding was associated with a high concentration of *Sinocalanus sinensis* in the stomachs of the fish. Therefore, if the growth and condition indices are coupled with recent feeding, it is reasonable to assume that the better condition was associated with a better feeding in the oligohaline region. Therefore, the spatial variability in growth and condition appears to be related not only to the spatial variability in diet quantity but also to the variation in diet quality, more particularly to the variability in trophic structure. Using the recent growth history as an indicator, Wainright et al. (1996) reported regionally variable habitat quality and showed that the maximal growth

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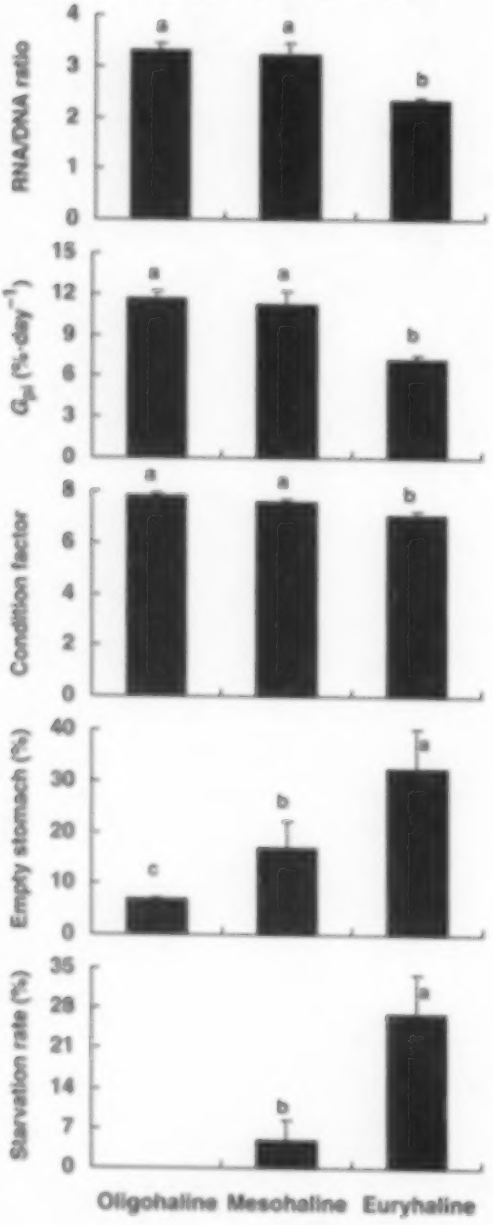
Fig. 9. Spatial variations in RNA/DNA ratio, protein growth rate (G_{pt}; %·day^{-1}), condition factor, empty stomachs (%), and starvation rates (%) of Japanese sea bass (*Lateolabrax japonicus*) larvae and juveniles in the oligohaline, mesohaline, and euryhaline regions along the Chikugo River estuary. The letters assigned to each value indicate significant differences ($P < 0.05$); values having different letters are significantly different. Data are mean + standard error from samples in March 2002 and 2003.



rate of juvenile striped bass corresponded with the turbidity maximum in the oligohaline region of the Delaware estuary. Using stable isotope ratios, Wainright et al. (1996) showed that the spatial variability in the growth rates of juvenile striped bass was related to the qualitative aspect of the diet.

Calculations of starvation status from theoretical estimates based on growth models of different species should be used with extreme caution, because critical values of RNA/DNA ratios are species-specific and show spatio-temporal and regional variabilities. Therefore, species calibration by laboratory experiment is needed to have concrete information on the critical RNA/DNA values during starvation, which is absent for Japanese sea bass. Although the calculation used in the present study is not an absolutely reliable estimate of starvation, we report a reliable scale for between-region comparison that showed that a higher proportion of the fish is starving in the euryhaline region. Moreover, our starvation estimates are in close agreement with the proportion of empty stomachs, which was significantly higher in the euryhaline region than in the oligohaline region.

Regional variations in all indices of the nursery functions (copepod dry biomass, gut content dry biomass, feeding intensity, RNA/DNA ratio, G_{pt}, condition factor, empty stomach, starvation rates) led us to believe that the oligohaline region is a better nursery habitat than the euryhaline region in the Chikugo River estuary. This differential habitat quality also points to the importance of the ascending movement of the sea bass. If, for example, the fish fails to reach the oligohaline nursery habitat, the fish would definitely experience even greater starvation and lower growth resulting perhaps from density-dependent food competition and poor feeding. This would result in serious effects on survival and recruitment of the fish and thus have critical demographic consequences. Therefore, it is reasonable to assume that the dispersion and ascending movement of the sea bass larvae and juveniles is linked to the feeding and nutritional history and more particularly to the spatial variability in the habitat quality and the nursery functions between the upper and the lower estuary.

Two contrasting spatial zones were identified along the estuary in terms of hydrographic variables, fish distribution, qualitative and quantitative feeding, fish larval growth and condition, qualitative and quantitative abundance of copepods, and qualitative and quantitative feeding of copepods. A zone of turbidity maximum exists in the oligohaline upper estuary, which is also characterized by nutrient and chlorophyll maxima. In the oligohaline region, *Sinocalanus sinensis*, an oligohaline calanoid copepod, contributes tremendously to fish diet. This copepod receives energy from a detrital source that supports significantly higher copepod biomass in the oligohaline region. In contrast, in the euryhaline region, fish diets are dominated by several common coastal copepods that receive energy from an algal source. Based on these dietary relationships, we identified two contrasting food webs in the two spatially contrasting regions in the estuary: (*i*) a detrital food web in the oligohaline upper estuary and (*ii*) an algal food web in the euryhaline lower estuary. The detrital food web appears to be associated with a more productive and nutritionally better ecosystem than the algal food web at the base of the system. The ETM positively influences growth and condition of fish larvae and juvenile through enhanced larval feeding and thus plays important role as nursery for many estuarine-dependent fishes.

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Validating species sensitivity distributions using salinity tolerance of riverine macroinvertebrates in the southern Murray–Darling Basin (Victoria, Australia)

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Abstract: Species sensitivity distributions (SSDs) are commonly used in risk assessment and in setting water quality guidelines, yet their predictions have not been validated against loss of species with increasing pollutant concentrations in nature. We used a rapid toxicity testing method to determine the acute salinity tolerance (72 h LC_{50} values (concentration of salinity lethal to 50% of individuals)) of 110 macroinvertebrate taxa from the southern Murray–Darling Basin in central Victoria, Australia, and construct an SSD. This SSD was compared with loss of riverine macroinvertebrates species from increasing salinity in Victoria. Macroinvertebrate species richness per individual sample, when salinity was <9.9 mS·cm^{-1}, was invariant of salinity. However, when species richness was calculated across multiple samples above about 0.3–0.5 mS·cm^{-1}, it declined with increasing salinity. This decline was predicted from the SSD after application of a variable safety factor calculated from an exponential or quadratic equation. Our findings confirm that SSDs can predict the loss of freshwater macroinvertebrate species from increases in salinity. This suggests that SSDs may be useful more generally for other aquatic organisms, other stressors, and toxicants.

Résumé : Les distributions de la sensibilité des espèces (SSDs) sont couramment utilisées pour l'évaluation des risques et l'établissement de normes de qualité de l'eau, bien que leurs prédictions n'aient jamais été validées à partir des pertes d'espèces en fonction des augmentations des concentrations de polluants en nature. Nous utilisons un test rapide de toxicité pour déterminer la tolérance à la salinité aiguë (valeurs de LC_{50}, soit la concentration de salinité létale pour 50 % des individus, après 72 h) de 110 taxons de macroinvertébrés du sud du bassin versant Murray–Darling dans le centre de l'état de Victoria, Australie, et nous construisons une SSD. Nous comparons la SSD à la perte d'espèces de macroinvertébrés de rivière en fonction d'une augmentation de la salinité dans l'état de Victoria. La richesse spécifique des invertébrés par échantillon individuel ne varie pas en fonction de la salinité lorsque cette dernière est <9,9 mS·cm^{-1}. Cependant, lorsque la richesse en espèces est calculée à partir d'échantillons multiples, elle décline de façon inversement proportionnelle à la salinité aux salinités supérieures à environ 0,3–0,5 mS·cm^{-1}. Ce déclin est prédit par la SSD après l'application d'un facteur de sécurité calculé à partir d'une équation exponentielle ou quadratique. Nos résultats confirment que les SSD peuvent prédire la perte d'espèces de macroinvertébrés en réaction à une augmentation de la salinité. Cela laisse donc croire que les SSD peuvent être utilisées de façon plus générale pour d'autres organismes aquatiques, pour d'autres facteurs de stress et d'autres produits toxiques.

[Traduit par la Rédaction]

Introduction

To assess risks from environmental pollution and derive ambient water quality guidelines for protecting biodiversity, many laboratory-based studies have been conducted on the effects of chemical contaminants on aquatic organisms. However, these effects are rarely compared with what occurs in nature. The compilation of laboratory toxicity data from multiple species to produce species sensitivity distributions (SSDs) is commonly used to derive water quality guidelines (e.g., ANZECC and ARMCANZ 2000; Warne 2001; Jooste and Rossouw 2002). It is also possible to produce SSDs from data on the concentration of a chemical where species are observed in nature (e.g., Hart et al. 2003); however, this is rarely done.

An estimate of the maximum concentration of a toxicant that should protect some percentage of species can be deduced from an SSD using a variety of statistical methods

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(e.g., Aldenberg and Slob 1993; Shao 2000; Grist et al. 2002) or can be empirically determined (Jones et al. 1999). If the concentration is kept below the concentration that will harm some percentage of species tested (in laboratory experiments), then no more than this percentage of species should be adversely affected in nature by this toxicant. This prediction does not necessary imply that the number of species present, or species richness, in a sample will decrease by the percentage deduced from the SSD. This is because the distributions of individual species are affected by many biotic and abiotic factors. Therefore, a particular species may be absent from a site because of biotic interactions; nontoxicant, abiotic influences (e.g., habitat); or a past disturbance that eliminated it from the site rather than the presence of a particular toxicant. Furthermore, direct comparisons of species richness between samples are not straightforward (Gotelli and Colwell 2001). The critical point is that predictions from SSD relates to the pool of species that can potentially be present at a site, rather than the species richness that is in fact present.

Such predictions have never been tested, and the use of SSD in risk assessments and water quality guidelines has thus not been validated. Such predictions may not hold because the SSD has not incorporated a response variable, or endpoint, that is of ecological relevance. In an attempt to overcome this, when SSDs are compiled from acute lethal tolerance data, it is necessary to use some extrapolation or safety factor to account for chronic and sublethal effects (Forbes and Calow 2002*a*). While a variety of safety factors have been suggested and used for different toxicants, their utility has not been validated with field data. Furthermore, the SSD approach assumes that toxicants have no indirect effects. Indirect effects occur when tolerant species are not affected directly by the toxicant, but more sensitive species being affected by the toxicant go on to affect the tolerant species, because these sensitive species are their prey, predators, competitors, parasites, etc.

To test such predictions against species richness trends in nature, we must assume that any relationship between the biota and the toxicant of interest is not caused by some other factor(s) that co-vary with the toxicant of interest. We return to this assumption in the discussion.

Until recently, one of the problems in applying SSDs has been the inability to obtain samples of tested species that are representative of natural communities (Kooijman 1987; Forbes and Calow 2002*b*). However, this problem can be overcome by the use of rapid toxicity tests (Kefford et al. 2005*a*), which are designed to quickly determine the approximate sensitivity of many species to some contaminant. Essentially, the rapid test approach involves the exposure of fewer individuals of a species and where a species is only obtainable in low numbers, as well as sequential exposure and the concurrent exposure of multiple species. While the estimates at the species level are approximate, by being able to test many species quickly, including rare species, and from a variety of taxa and functional groups, the species tested can be more representative of the species present in nature (Kefford et al. 2005*a*).

Rapid toxicity tests were used to derive 72 h concentration of salinity lethal to 50% of individuals (LC_{50}) for freshwater macroinvertebrates collected from rivers in the southern Murray–Darling Basin in cental Victoria, Australia. This data set is used to construct an SSD, which is then compared, with and without various safety factors, with the loss of Victorian riverine macroinvertebrate species richness with increasing salinity. We stress that this study was conducted in Victorian rivers that are or were fresh water or only slightly saline and where high salinities are largely the result of anthropogenic changes. In estuaries and marine systems, the response of organisms to salinity will likely differ to that reported here. In southwest Australia, there is a longer history of primary (natural) and secondary (anthropogenic) salinization, and inland aquatic macroinvertebrates would appear to have greater salinity tolerance than those from eastern Australia (see Kay et al. 2001; Pinder et al. 2005). Therefore, the specific levels of salinity that induces changes to macroinvertebrate communities may differ between eastern and western Australia.

While salinity has not traditionally been considered a toxicant, its effects on freshwater species can be considered similar to that of essential elements (Kefford et al. 2002), and there are a number of advantages of using salinity to validate SSDs. Salinity and its component ions are conservative, and bioaccumulation, biotransformation, and bioavailability are probably not issues as they are with many traditional toxicants. Salinity is also very simple to measure (see Williams and Sherwood 1994) relative to other contaminants, resulting in large biotic data sets with concurrent salinity measurements. Furthermore, rising salinity is a very significant environmental contaminant in many rivers and wetlands in Australia and elsewhere (Williams 1987), and studying its effects on freshwater organisms is important in its own right (Williams 1987; Hart et al. 1991; MDBMC 1999).

Materials and methods

Toxicology

Macroinvertebrates were collected from six sites in the Goulburn, Broken, Campaspe, and Loddon river catchments in central Victoria, Australia, in the southern Murray–Darling Basin (Table 1). This area spans the rainfall range of 600–1200 mm·year^{-1} falling mostly in winter and spring. The electrical conductivity (EC) at the time of collections ranged from 0.03 to 1.6 mS·cm^{-1} (Table 1). All EC values were corrected to 25 °C. As laboratory tests were conducted in a nonflowing environment, macroinvertebrates were only collected from slow-flowing sections of the river. Macroinvertebrates were transferred to the laboratory in the water from which they were collected.

The rapid toxicity testing methods used here are identical to those used by Kefford et al. (2003*a*, 2005*b*) and discussed by Kefford et al. (2005*a*) and thus are only briefly described here. Salt solutions were prepared by dissolving Ocean Nature (Aquasonic, Wauchope, NSW, Australia) sea salt in dechlorinated and filtered Melbourne tap water (~0.1–0.15 mS·cm^{-1}), hereafter referred to as wet lab water. Macroinvertebrates were taken to the laboratory and randomly allocated to their respective treatments after the collection water reached the approximate temperature of the test water (20 °C). No other acclimation was conducted. Mortality was assessed every 24 h.

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Table 1. Information on the macroinvertebrate collection sites.

Site	Latitude, longitude	EC range*
King Parrot Creek at Flowerdale (Goulburn Catchment)	37°23′S, 145°16′E	0.06–0.10
Campaspe River at Kyneton–Heathcote Road	37°23′S, 144°31′E	0.07–1.60
Sunday Creek at Deckerys Road (Goulburn Catchment)	37°10′S, 145°06′E	0.08–1.50
Broken River at Bridge Creek Road	37°13′S, 146°17′E	0.03–0.22
Goulburn River at Gardiners Road	37°20′S, 145°18′E	0.06
Loddon River at Pyrenees Highway	37°07′S, 144°05′E	0.39–0.62

*Electrical conductivity (EC, $mS \cdot cm^{-1}$) range refers to when the macroinvertebrates were collected.

All tests were run for at least 72 h to be consistent with other acute salinity tolerance experiments with macroinvertebrates (Kefford et al. 2003*a*, 2005*b*); a subset was extended to 96 h. On death or at the completion of the experiment, all organisms were preserved for subsequent identification to species level where practical. While it was not possible to identify all animals to species level (Table 2), this situation also existed with the species richness data. Related freshwater macroinvertebrates tend to have similar acute lethal salinity tolerance (Kefford et al. 2003*a*, 2005*b*); we thus maintain that some non-species-level identification would have minimum effect on the study findings.

In this paper, salinity will be expressed in terms of EC. For comparisons with results in other units, the relationships for Ocean Nature are as follows: TDS = 0.754 × EC and osmolarity = 0.0184 × EC (Kefford et al. 2003*a*), where TDS is total dissolved solids in $g \cdot L^{-1}$, EC is in $mS \cdot cm^{-1}$ at 25 °C, and osmolarity is in osmol·kg H_2O^{-1}.

For common species, arbitrarily defined as those with ≥50 individuals collected on at least one occasion, LC_{50} values were calculated from logistic regression equations. For rare species, or those with <50 individuals collected on all occasions, LC_{50} values were empirically determined (Kefford et al. 2003*a*, 2005*b*). For rare species, the LC_{50} values were estimated as a point, a range, or greater than some value (censored). Where LC_{50} values were estimated as a range, it was assumed to be the midpoint of this range. Given the presence of censored LC_{50} values, the SSD was calculated empirically using the Kaplan–Meier method (Smith 2002). This method is conservative and includes censored data in its calculations (see Kefford et al. 2003*a*, 2005*a*, 2005*b*).

From the Kaplan–Meier function, protective concentrations (PCx) to protect various proportions of species were determined. Elsewhere, preliminary results suggested that sublethal effects of high salinity for macroinvertebrates occurred at 10%–30% of their 72 h LC_{50} value (Kefford et al. 2003*b*). We thus calculated PCx values from the Kaplan–Meier function after applying 10%, 20%, and 30% safety factors. We also applied a 5% safety factor to be conservative.

Species richness – EC relationship in nature

All edge samples with species-level identification were extracted from the Victorian Environment Protection Authority (EPA) macroinvertebrate database. This resulted in 1597 samples collected between 1990 and 2004 from 379 sites throughout Victoria over a salinity range 0.015–35.5 $mS \cdot cm^{-1}$. The majority of the samples (73%) came from reference sites (defined as the least disturbed of their type available), with the remaining 27% from test sites (whose quality is unknown). All samples were collected using a standardized, qualitative sampling protocol for macroinvertebrates and associated environmental data, including EC (Tiller and Metzeling 1998; Davies 2000). Samples were collected with a sweep net (mesh size 0.25 mm) at least twice from each site in spring (October–December) and autumn (March–May), with live sorting of specimens on site followed by laboratory identification. During live sorting, the aim was to collect as many different taxa as possible, and for most (>95%) samples it was conducted for 30 person minutes. When new taxa were found in the last 5 min, sorting continued for a further 10 min. If in this additional time further new taxa were found, further 10 min intervals were added, up to a maximum of 60 min of total sorting. The additions of 10 min intervals have minimal effect on the total number of individuals collected per sample (L. Metzeling, personal observations). We only used samples from edge habitats because the other habitats regularly sampled by the EPA (riffles) are generally rare in saline Victorian rivers, and including riffle habitats would confound any comparison between samples of different salinities. Furthermore the toxicity data was for macroinvertebrates collected from the edge habitat. Data from the EPA database has previously been published (e.g., Marchant et al. 1997, 1999; Kefford et al. 2004*a*).

Most samples in the EPA database are from a low-salinity environment (<1 $mS \cdot cm^{-1}$). Consequently, a direct comparison of species richness between EC ranges (or categories) would be biased by the number of samples in each category (see Gotelli and Colwell 2001). We therefore selected EC categories with a wider range as EC increased. Even then it was not possible to select EC categories that both contained a similar number of samples in each category and represented useful EC ranges from which to compare the toxicological data. After excluding the highest EC category (15–36 $mS \cdot cm^{-1}$), which contained only one sample, the category with the fewest samples (10–14.9 $mS \cdot cm^{-1}$) contained 12 samples. We therefore considered species richness from 12 samples using two rarefaction methods. (We also considered species richness from one sample, to allow comparisons with previous studies.) Firstly, we randomly selected, without replacement, sets of 12 samples from each category and counted the number of species per 12 samples. Where ≥36 samples were in a category, we determined the mean and 95% confidence intervals (CI). Secondly, we used the procedure described by Colwell et al. (2004) to calculate the number of species observed (S_{obs}) at 1–12 samples, using the software program EstimateS 7.5 (Colwell 2005). This procedure is robust to a number of as-

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Table 2. LC_{50} values for macroinvertebrates tested from the southern Murray–Darling Basin.

Taxa	Abundance	LC_{50} value ($mS \cdot cm^{-1}$) 72 h	96 h	Total no. tested for 72 h value
Chironomus occi (Diptera: Chironomidae)	Rare	>0.1		2
Diamesinae sp. (Diptera: Chironomidae)	Rare	>0.1	>0.1	1
Pentaneurini (Diptera: Chironomidae)	Rare	>0.1		1
Anisitsiellides sp. (Acarina: Anisitsiellidae)	Rare	>0.1	>0.1	1
Coaustraliobates longipalps (Acarina: Hygrobatidae)	Rare	>0.1	>0.1	1
Aedes (Ochlerotatus) sp. (Diptera: Culicidae)	Rare	>0.23	>0.23	1
Macrogyrus reichei (Aube) (Coleoptera: Gyrinidae)	Rare	>3.2	>3.2	1
Orthetrum caledonicum (Odonata: Libellulidae)	Rare	>3.2		2
Recifella sp. (Arachnida: Unioncolidae)	Rare	>3.2		2
Anopheles sp. (Diptera: Culicidae)	Rare	>6.4		7
Branchiura sowerbyii (Oligochaeta: Tubificidae)	Rare	>6.4		1
Chironomus alternans (Diptera: Chironomidae)	Rare	>6.4		1
Chironomus sp. (Diptera: Chironomidae)	Rare	>6.4		6
Gretacarus sp. (Acarina: Mideopsidae)	Rare	>6.4	>6.4	1
Halomicrus sp. (Isopoda: Oniscidae)	Rare	>6.4		1
Cura sp. (Turbellaria: Tricladida)	Rare	3.2–12.8		2
Glyptophysa aliciae (Gastropoda: Planorbidae)	Rare	6.4–12.8	6.4–12.8	34
Stempoellina sp. (Diptera: Chromonidae)	Rare	0.1–20		2
Glyptophysa gibbosa (Gastropoda: Planorbidae)	Rare	3.2–20		3
Physa acuta (Gastropoda: Physidae)	Common	12.6		378
Atalophlebia sp. (Ephemeroptera: Leptophlebiidae)	Rare	12.8		5
Coquillettidia sp. (Diptera: Culicidae)	Rare	>12.8		1
Diaprepocoris sp. (Hemiptera: Corixidae)	Rare	>12.8		6
Glossiphonia australiensis (Hirudinea: Glossiphoniidae)	Rare	>12.8	>12.8	1
Glossiphonia sp. (Hirudinea: Glossiphoniidae)	Rare	>12.8		5
Parastenotarsus grimmii (Diptera: Chironomidae)	Rare	12.8		19
Procordulia jacksoniensis (Odonata: Corduliidae)	Rare	>12.8		2
Sclerocyphon sp. (Coleoptera: Psephenidae)	Rare	>12.8		1
Stenochironomus watsonii (Diptera: Chironomidae)	Rare	>12.8		1
Pentaneurini sp. (Diptera: Chiromonidae)	Rare	>12.8	>12.8	3
Triplectides ciuskus (Trichoptera: Leptoceridae)	Rare	>12.8		4
Orthocladiinae sp. (Diptera: Chiromonidae)	Rare	>12.8	>12.8	1
Atalophlebia AV 5 (Ephemeroptera: Leptophlebiidae)	Rare	12.8–15	12.8–15	36
Semilagenta hilli (Hirudinea: Glossiphoniidae)	Rare	12.8–15		10
Aeshna brevistyla (Odonata: Aeshnidae)	Rare	>15		1
Atalophlebia sp. AV 9 (Ephemeroptera: Leptophlebiidae)	Rare	>15		8
Austropeplea tomentosa (Gastropoda: Lymnaeidae)	Rare	15		10
Centroptilum–Cloeon sp. (Ephemeroptera: Baetidae)	Rare	15	12.8–15	62
Chironomus occidentalis (Diptera: Chironomidae)	Rare	>15		4
Coquillettidia sp. 2 (Diptera: Culicidae)	Rare	15		6
Hellyethira sp. (Trichoptera: Hydroptilidae)	Rare	>15	>15	5
Paramerina levidensis (Diptera: Orthocladiinae)	Rare	>15		1
Sigara subtaevifrons (Hemiptera: Corixidae)	Rare	>15		41
Triplectides sp. (Trichoptera: Leptoceridae)	Rare	>15		1
Limnesia corpulenta (Acarina: Limnesiidae)	Rare	6.4–25	6.4–25	38
Lumbriculus variegatus (Oligochaeta: Lumbriculidae)	Rare	15–20	15	21
Enithares sp. (Hemiptera: Notonectidae)	Rare	6.4–30		2
Dasyhelea EPA sp. 43 (Diptera: Ceratopogonidae)	Rare	12.8–25		2
Tanytarsus spp. (including *fuscithorax*) (Diptera: Chrominidae)	Rare	12.8–25		4
Antiporus femoralis (Boh.) (Coleoptera: Dytiscidae)	Rare	3.2–35		2
Agraptocorixa eurynome (Hemiptera: Corixidae)	Rare	15–25		10
Chironomus cloacalis (Diptera: Chironomidae)	Rare	12.8–20	>12.8	33
Amphiops sp. (Coleoptera: Hydrophilidae)	Rare	>20		1
Atalophlebia sp. 13 (Ephemeroptera: Leptophlebiidae)	Rare	>20	>20	5
Geocherax sp. (Decapoda: Parastacidae)	Rare	>20		1

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Table 2 (*concluded*).

Taxa	Abundance	LC_{50} value ($mS \cdot cm^{-1}$) 72 h	96 h	Total no. tested for 72 h value
Hyphydrus elegans (Coleoptera: Dyticidae)	Rare	>20		6
Lancetes lanceolatus (Coleoptera: Dyticidae)	Rare	>20		1
Mesovelia hungerfordi (Hemiptera: Mesoveliidae)	Rare	>20	15–20	9
Sphaerium sp. (Bivalvia: Sphaeriidae)	Rare	>20		2
Tasmanocoenis sp. (Ephemeroptera: Caenidae)	Rare	>20	>20	2
Micronecta robusta (Hemiptera: Corixidae)	Common	21.5		791
Archaeophylax canarus (Trichoptera: Limnephilidae)	Rare	15–30	15–30	2
Rheumatometra philarete (Hemiptera: Gerridae)	Rare	20–25		26
Notalina sp. (Trichoptera: Leptoceridae)	Common	23.7		317
Diphlebia coerulescens (Odonata: Diphlebiidae)	Rare	>25		1
Helochares sp. (Coleoptera: Hydrophilidae)	Rare	>25	>25	1
Hydrodroma sp. (Acarina: Hydrodromatidae)	Rare	>25	>25	4
Microvelia peramoena (Hemiptera: Veliidae)	Rare	20–30		26
Notalina spira (Trichoptera: Leptoceridae)	Rare	20–30	20–30	36
Sigara australis (Hemiptera: Corixidae)	Rare	20–30	15–30	20
Triplectides volda (Trichoptera: Leptoceridae)	Rare	>25		16
Eylaidae (Arachnida)	Rare	>25	>25	1
Koenikea sp. (Arachnida: Unionicolidae)	Rare	3.2–47		3
Triplectides similis (Trichoptera: Leptoceridae)	Rare	3.2–47	>35	2
Limnesia corpulenta (Acarina: Limnesiidae)	Rare	26.1		38
Leptocerus souta (Trichoptera: Leptoceridae)	Common	26.2		192
Pionа cumberlandensis (Acarina: Pionadae)	Rare	25–30		42
Oxus sp. (Acarina: Oxidae)	Rare	29.1		14
Paratya australiensis (Decapoda: Atyidae)	Common	29.1		201
Aeschna brevistyla (Odonata: Aeshnidae)	Rare	>30		1
Anisops sp. (Hemiptera: Notonectidae)	Rare	>30		1
Austrogomphus guerini (Odonata: Gomphidae)	Rare	>30		1
Bidessodes sp. (Coleoptera: Dytiscidae)	Rare	>30	>20	4
Chostonectes gigas (Boh.) (Coleoptera: Dyticidae)	Rare	>30		1
Diaprepocoris barycephala (Hemiptera: Corixidae)	Rare	30		3
Haliplus testudo Clark (Coleoptera: Haliplidae)	Rare	>30		1
Hemicordulia tau (Odonata: Hemicorduliidae)	Rare	>30		1
Necterosoma undecimlineatus (Bab.) (Coleoptera: Dyticidae)	Rare	>30		1
Platynectes decempunctatus (Fab) (Coleoptera: Dyticidae)	Rare	>30	>30	1
Scirtidae (Coleoptera)	Rare	>30	>30	2
Antiporus femoralis (Boh.) (Coleoptera: Dytisicidae)	Rare	30–35		17
Hydrachna sp. (Acarina: Hydrachnidae)	Rare	30–35	30	19
Enithares woodwardi (Hemiptera: Notonectidae)	Rare	35	25	52
Eretes australis (Coleoptera: Dytiscidae)	Rare	>35	>35	1
Helochares tristis (MacLeay) (Coleoptera: Hydrophilidae)	Rare	>35	>35	3
Antiporus blakei (Coleoptera: Dytiscidae)	Rare	25–47		19
Byrrocryptus sp. (Coleoptera: Ptilodactylidae)	Rare	30–47		2
Naucoris congrex (Hemiptera: Naucoridae)	Rare	30–50		17
Austrolestes psyche (Odonata: Lestidae)	Rare	35–47	35–47	3
Necterosoma penicillatum (Coleoptera: Dyticidae)	Common	43.9		99
Arrenurus balladoniesis (Acarina: Arrenuridae)	Rare	35–55	25–55	7
Xanthagrion erythroneurum (Odonata: Coenagrionidae)	Rare	45.7		30
Berosus majusculus Blackb. (Coleoptera: Hydrophilidae)	Rare	>47		4
Hydrochus sp. (Coleoptera: Hydrochidae)	Rare	>47	>47	2
Ischnura aurora (Odonata: Coenagrionidae)	Rare	47		26
Nososticta solida (Odonata: Protoneuridae)	Rare	>47	>6.4	2
Synlestes weyersii (Odonata: Synlestidae)	Rare	47		7
Austrochiltonia subtenuis (Amphipoda: Ceinidae)	Common	49		252

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sumptions that other rarefaction methods make (see Colwell et al. 2004).

Comparing species richness – EC relationship with SSD

We compared the loss in species richness across a number of samples in each EC category with the percentage of species whose 72 h LC_{50} values were exceeded from the SSD. Species richness per 12 samples and S_{obs} values from 12 samples (hereafter $S_{obs}(12)$) were similar. Thus, comparisons between the SSD and species richness were only made with $S_{obs}(12)$ values. $S_{obs}(12)$ values were rescaled as a percentage of the EC category with the maximum $S_{obs}(12)$ values (which was the 0.10–0.19 mS·cm^{-1} category having an $S_{obs}(12)$ value of 162 species), hereafter $S_{obs}(12)$ percentages. These $S_{obs}(12)$ percentages were then compared with concentrations calculated from the SSD that should protect various percentages of taxa (i.e., PCx values) with no safety factor and with 5%, 10%, 20%, and 30% safety factors applied to the SSD (i.e., we wished to test the hypothesis that there is no difference in the observed difference in $S_{obs}(12)$ percentages relative to EC and the decreases predicted by the SSD with no safety factor or one of the above safety factors). If the SSD with a particular safety factor matched the percentage of species richness in nature, then this predicted loss of species should match the actual loss in the $S_{obs}(12)$ percentages.

Statistical comparisons

Differences in the mean number of species per 12 samples between EC categories were evaluated by a single factor analysis of variance (ANOVA) followed by a post hoc Tukey's honestly significance difference (HSD) test. Assumptions of normal distribution and equal variance were confirmed by viewing boxplots. Statistical differences in S_{obs} values between EC categories were determined by nonoverlapping 95% CI as calculated in EstimateS 7.5 (Colwell 2005).

To explore possible links between $S_{obs}(12)$ percentages and the SSD's predictions (other than these predictions being reduced by a simple percentage), various nonlinear regressions models representing several curve shapes (including exponential, quadratic, logarithmic, and S) were fitted for the subset of $S_{obs}(12)$ percentages, which have SSD values with approximately the same percentage loss of taxa ($n = 5$) using the software SPSS version 13 (SPSS Inc., Chicago, Illinois). The two best models were judged in terms of r^2 and not under- or over-estimating values throughout the data range.

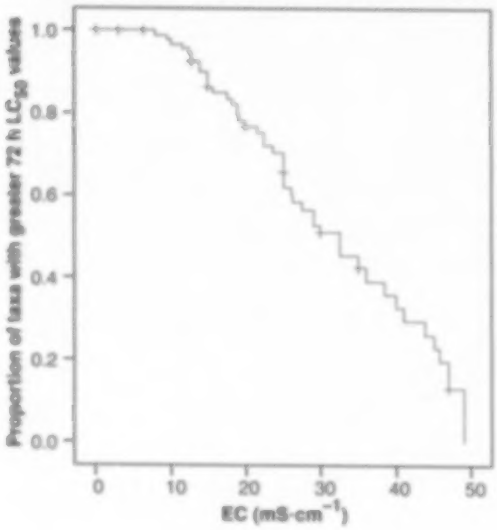
Results

Toxicology

Acute lethal salinity tolerance data were obtained for 110 taxa (Table 2) and the Kaplan–Meier function of 72 h LC_{50} values established (Fig. 1), which shows that after the most sensitive, noncensored species (72 h LC_{50} values ~10 mS·cm^{-1}), there is an approximately linear decrease in more tolerant species.

The choice of safety factor is crucial to determine the critical EC value (Table 3). For example, to protect 95% of species with a 5% safety factor, the critical EC is 0.58–0.63 mS·cm^{-1}, but with a 30% safety factor it is 3.5–3.8 mS·cm^{-1}.

Fig. 1. Kaplan–Meier function of 72 h LC_{50} values (lethal concentration for 50% of individuals) for macroinvertebrates from the southern Murray–Darling Basin; crosses in survival function indicate censored LC_{50} values. EC, electrical conductivity.



Species richness – EC relationship in nature

Species per 12 samples

There was a significant difference in the number of species per 12 samples among the various EC categories (Table 4: $F = 7.2$, df = 8 and 110, $P < 0.001$, including categories with >2 replicates; see Fig. 2). All EC categories < 0.50 mS·cm^{-1} have a similar number of species per 12 samples (Fig. 2), but the number of species in the 0.50–0.99 mS·cm^{-1} category is significantly lower than that in most of the previous categories (Fig. 2). The number of species per 12 samples in the 0.50–0.99 mS·cm^{-1} category was 92% of that in the richest category (0.20–0.29 mS·cm^{-1}) (Table 4).

Species observed (S_{obs})

Colwell et al.'s (2004) incidence-based rarefaction procedure showed a similar S_{obs} in one sample ($S_{obs}(1)$ value) in each of the EC categories (Table 4; Fig. 3). There was a significantly lower $S_{obs}(1)$ value in the 10–14.9 mS·cm^{-1} category than in the 0.05–0.09 mS·cm^{-1} category; also the one sample in the 15–36 mS·cm^{-1} category only had 10 species (Table 4). However, given the lack of replication in this category, it is not possible to assess its statistical significance. Whatever the case, given the variation within categories, at <9.9 mS·cm^{-1}, species richness per sample would appear to be invariant of salinity.

However, as the number of samples over which S_{obs} are calculated increases, differences in species richness among EC categories become apparent (Fig. 3). These differences are clearer when scaled for number of individual organisms (see Gotelli and Colwell 2001). By $S_{obs}(12)$, there were clear differences among EC categories (Fig. 3; Table 4). While $S_{obs}(12)$ values were similar (≈150) in all EC categories ≤0.49 mS·cm^{-1} (Table 4), more individual organisms were collected to reach approximately the same S_{obs} value as EC

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Table 3. Protective concentrations of salinity (mS·cm^{-1}) for selected percentages of taxa with various safety factors.

	Safety-application factor				
Percentage of taxa protected (SE)*	5%	10%	20%	30%	100% (no safety factor)
98.9% (1.1)	0.40	0.80	1.6	2.4	8.0
95.6% (2.1)	0.58	1.2	2.3	3.5	11.6
94.6% (2.4)	0.63	1.3	2.5	3.8	12.6
92.4% (2.8)	0.64	1.3	2.6	3.8	12.8
89.9% (3.2)	0.69	1.4	2.8	4.2	13.9
82.1% (4.2)	0.91	1.8	3.6	5.5	18.2
79.4% (4.5)	0.94	1.9	3.8	5.7	18.9
50.9% (6.3)	1.5	3.0	6.0	9.0	30.0
19.6% (6.7)	2.3	4.6	9.1	14	45.7

*Standard error is given in parentheses.

Table 4. Summary of species richness measures for each of the electrical conductivity (EC) categories.

EC category (mS·cm^{-1})	No. of samples	Species per 12 samples (% of maximum)	$S_{obs}(1)$ (95% CI)	$S_{obs}(12)$ (95% CI)	$S_{obs}(12)$ (% of maximum)
<0.050	296	97	23.4 (19.9–26.8)	156 (141–172)	97
0.050–0.099	338	100	25.9 (21.8–29.9)	161 (145–176)	99
0.10–0.19	232	99	24.4 (20.7–28.2)	162 (147–176	100
0.20–0.29	130	100	25.1 (21.1–29.1)	160 (146–174)	99
0.30–0.49	113	96	24.5 (20.6–28.4)	156 (142–169)	96
0.50–0.99	141	92	23.4 (19.5–27.3)	147 (134–160)	91
1.0–1.49	88	82	22.5 (18.3–26.7)	134 (121–146)	83
1.5–1.9	73	78	20.7 (16.7–24.6)	128 (115–141)	79
2.0–2.9	63	83	22.8 (18.5–27.2)	137 (124–151)	85
3.0–3.9	30	68	20.2 (16.2–24.3)	117 (106–129)	73
4.0–4.9	21	71	22.0 (17.0–27.0)	115 (103–128)	71
5.0–6.9	32	73	22.8 (17.7–27.9)	117 (104–130)	73
7.0–9.9	21	68	21.3 (16.6–26.0)	112 (101–123)	69
10–14.9	12	51	17.3 (13.4–21.3)	99.0 (84.1–114)	61
15–36	1	—	10	—	—

Note: S_{obs}, the number of species observed; CI, confidence interval.

increased (Fig. 3). For example, the $S_{obs}(12)$ value for both the <0.050 and 0.3–0.49 mS·cm^{-1} categories was 156 (Table 4); these categories had a mean of 1852 and 3089 individuals, respectively, recorded in 12 samples.

At higher EC categories, there was a general trend for $S_{obs}(12)$ values to decrease with increasing salinity (Table 4; Fig. 3). The $S_{obs}(12)$ value in the 1.0–1.49 mS·cm^{-1} category was significantly less than the values in the categories 0.10–0.19 and 0.20–2.9 mS·cm^{-1} (Table 4). With the exception of the 1.5–1.9 mS·cm^{-1} category, all EC categories >2.0–2.9 mS·cm^{-1} had significantly lower $S_{obs}(12)$ values than EC categories ≤0.3–0.49 mS·cm^{-1} (Table 4). In categories >1.0–1.49 mS·cm^{-1}, $S_{obs}(12)$ values relative to the number of individuals collected generally fell with increasing EC category (Fig. 3). This was not the case in categories from 1.0–1.49 to 4.0–4.9 mS·cm^{-1}, where the trend in $S_{obs}(12)$ values was not related to EC.

Comparing species richness – EC relationship with SSD

$S_{obs}(12)$ values were expressed as a percentage of its maximum (recorded at 0.10–0.19 mS·cm^{-1}) in each EC category (Table 4). The reduction in these $S_{obs}(12)$ percentages approximated the loss of species predicted by the SSD with a 5%–10% safety factor over the EC range 0.10–1.9 mS·cm^{-1} (Fig. 4). It is also apparent that the SSD with a 5% safety factor is underprotective of $S_{obs}(12)$ percentages in the 0.10–0.19 and 0.20–0.29 mS·cm^{-1} EC categories. In the 0.30–0.49 and 0.50–0.9 mS·cm^{-1} categories, the $S_{obs}(12)$ percentage sits between the SSD's predictions with 5% and 10%; in the 1.5–1.9 mS·cm^{-1} category, the $S_{obs}(12)$ percentage approximately fits the SSD's prediction with a 10% safety factor. At EC > 1.5–1.9 mS·cm^{-1}, it is necessary for the SSD to have a safety factor somewhat >10% to match the drop in $S_{obs}(12)$ percentage. Therefore, as salinity increases, a smaller reduction in the SSD would appear to be needed so that its predictions match the $S_{obs}(12)$ percentage. The $S_{obs}(12)$ percentage and the loss of species predicted from the SSD may thus be better linked with a more complex function than a simple percentage of the SSD's predictions.

To explore other possible links between $S_{obs}(12)$ percentages and the SSD's predictions, nonlinear regressions models were fitted for the subset of $S_{obs}(12)$ percentages that have SSD values with approximately the same percent loss of taxa (n = 5). The best fits were found with exponential and quadratic regressions models (Fig. 5), with the regression equations described by

$$(1) \qquad EC_{S_{obs}(12)} = 0.0103\,EC_{SSD}^{2} - 0.152\,EC_{SSD} + 0.812$$

(adjusted r^2 = 0.92)

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Fig. 2. Mean and 95% confidence interval of number of species per 12 samples in each electrical conductivity (EC) category with greater than two replicates. EC categories with the same lower case letter are not statistically different from each other at the 0.05 level.

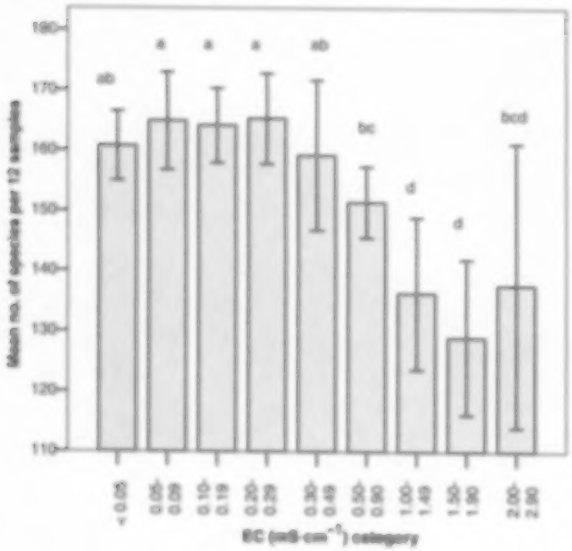
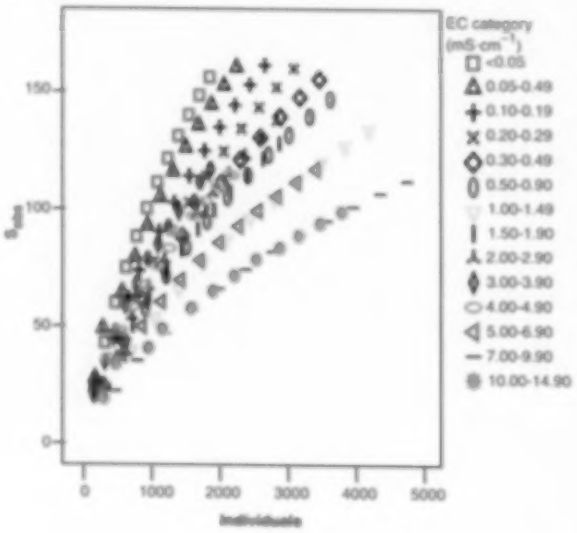


Fig. 3. Species rarefaction curves for 1–12 samples in each electrical conductivity (EC) category scaled to the number of individual organisms. For each EC category, 12 points are plotted representing S_{obs} (the number of species observed) values for 1–12 samples.



$$EC_{S_{obs}(12)} = 0.0601 \exp(0.173\, EC_{SSD}) \quad (2)$$

(adjusted $r^2 = 0.97$)

where $EC_{S_{obs}(12)}$ is the EC (mS·cm^{-1}) for a given reduction in the $S_{obs}(12)$ percentage (Table 4). EC_{SSD} is EC with the same percentage of taxa having their 72 h LC_{50} value exceeded from the SSD (Fig. 1; Table 2).

Adjusting the SSD with quadratic or exponential safety factors (eqs. 1 and 2, respectively) makes the predicted loss of species from the SSD fit the reduction in $S_{obs}(12)$ percentage up to the maximum EC category used here (i.e., 10–14.9 mS·cm^{-1}) (Fig. 6). Applying the exponential function to the SSD tracks the $S_{obs}(12)$ percentages slightly better than the quadratic function. Except when the SSD suggests that only 19.6% of taxa would be protected, the exponential function increases the SSD value from 45.7 to 166 mS·cm^{-1}. A safety factor should decrease the EC where a particular loss of species is expected, and caution should thus be applied if the safety factors are to be used at greater salinity than the maximum EC category in our study (10–14.9 mS·cm^{-1}).

Discussion

The SSD for 72 h LC_{50} values for macroinvertebrates from the southern Murray–Darling Basin was broadly similar to those for the Barwon River Catchment, Australia (Kefford et al. 2003*a*) and the southeast region of the Eastern Cape, South Africa (Kefford et al. 2005*b*). Considering $S_{obs}(1)$ values at salinity <9.9 mS·cm^{-1}, there were no detectable differences among the EC categories relative to the variation within these categories. However, as the number of samples increased, it is apparent that differences in S_{obs} values emerge among the different EC categories. Therefore, while the species richness per sample is similar in each of the EC categories, as salinity increases there is a tendency for a similar but smaller suite of species to be present in each sample. This implies that as the EC increases, there is a reduction in the pool of species that occur. After applying a simple mathematical function, either exponential or quadratic, as a safety factor, the reduction in species predicted by the SSD matched the reduction in $S_{obs}(12)$ percentage up to the highest EC category investigated (10–14.9 mS·cm^{-1}). In most cases, managers would want to protect the majority of species (≥90%), and salinities >15 mS·cm^{-1} are currently relatively rare in Victorian rivers. Therefore, having an SSD with a safety factor tracing the reduction in species richness up to 15 mS·cm^{-1} does not limit the usefulness of SSD and its safety factors.

It is not surprising that both exponential and quadratic functions produced similar safety factors, because it is possible to approximate an exponential function with a quadratic function (Glantz and Slinker 1990). Exponential functions, however, have a greater base in biological theory and have one more degree of freedom than quadratic functions. We observed a slightly better match of the $S_{obs}(12)$ percentage and the SSD adjusted with the exponential function than with the quadratic function. Therefore, it is probably better to use the exponential safety function.

Over large salinity ranges (from fresh water to hypersaline (≥65 mS·cm^{-1})), many studies have found a negative correlation between species richness per unit sample and salinity (e.g., Williams et al. 1990; Williams 1998; Pinder et al. 2005). However, across samples from fresh water to slightly saline (up to ~10–15 mS·cm^{-1}), most studies report that while community structure is related to salinity, species richness is either not or is poorly related to salinity. Using a sub-

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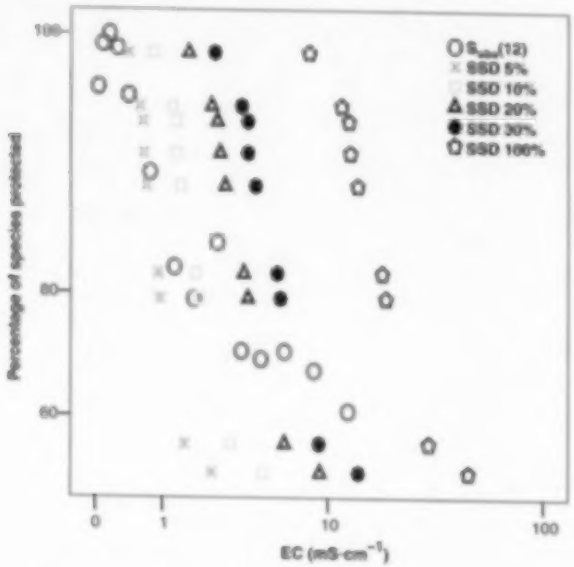
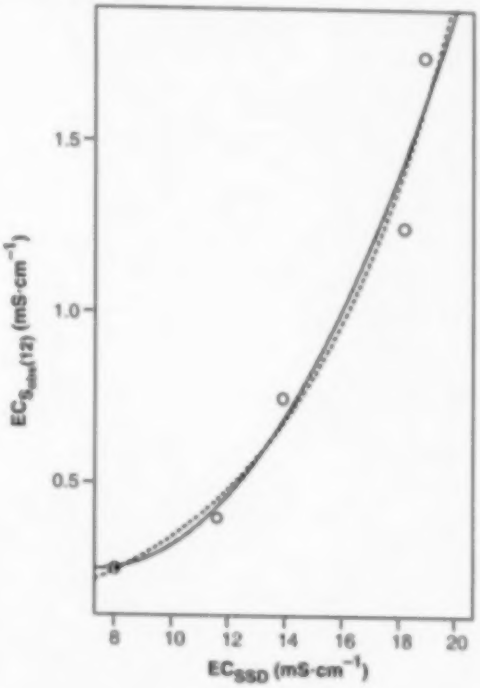
Fig. 4. $S_{obs}(12)$ (the number of species observed in 12 samples) percentages in each of the electrical conductivity (EC) categories (the midpoint of the categories is plotted) compared with the predicted loss of taxa from the species sensitivity distribution (SSD) with various safety factors. Note the nonlinear scales of the *x* and *y* axes.



set of the data used here, Marchant et al. (1997, 1999) found that while salinity was associated with the macroinvertebrate community, there is no relationship between salinity and species richness per sample. Using different data sets, other studies have found relationships between macroinvertebrate community structure and salinity but not between salinity and species richness per sample unit (Metzeling 1993; Kefford 1998*a*; Griffith et al. 2001). While Pinder et al. (2005) found that total macro- and micro-invertebrate species richness per wetland did not commence declining until 4.1 g·L^{-1} (~5.4 mS·cm^{-1}), this decline would probably not have been detected had they not sampled sites across a wide salinity gradient. Piscart et al. (2005) found a decline in species richness per site between 0.7 and 3.4 mS·cm^{-1}. Their comparison was based on the amalgamation of 9–10 samples taken from 6–7 habitat types per site, and thus the detection of a decline in species richness may be due to the use of multiple samples.

The conventional explanation for the lack of a relationship between species richness per sample and salinity over a narrow range is that salinity changes are insufficient, relative to variation in other abiotic and biotic factors, to affect species richness (Williams et al. 1990; Williams 1998). However, comparing species richness without reference to the species sampling curve is problematic, as measured species richness can differ because of differences in the underlying species richness, differences in the shape of the relative abundance distributions, or differences in the number of individuals examined (Gotelli and Colwell 2001). Gotelli and Colwell (2001) refer to species richness per sample unit as species density (the number of species per unit area) and stress that

Fig. 5. The best-fit, nonlinear regressions models showing the subset of $S_{obs}(12)$ (the number of species observed in 12 samples) percentages that have species sensitivity distribution (SSD) values with approximately the same percent loss of taxa. The solid line shows the quadratic model (eq. 1) and the dashed line shows the exponential model (eq. 2) that best fit $EC_{S_{obs}(12)}$ and EC_{SSD}. ($EC_{S_{obs}(12)}$ is the electrical conductivity (mS·cm^{-1}) for a given reduction in the $S_{obs}(12)$ percentage (Table 4). EC_{SSD} is EC with the same percentage of taxa having their 72 h LC_{50} value exceeded from the SSD.) Circles indicated the observed data points (n = 5).



it should not be confused with species richness, which they use for the number of species in some community, area, habitat, etc., of interest. In this paper, we showed that although the number of species per sample (i.e., $S_{obs}(1)$ value) was essentially invariant of salinity, species richness across EC categories decreased with increasing salinity. A reevaluation of previous data sets is needed to see if similar patterns are repeated elsewhere.

Despite the general decline in species richness from multiple samples with increasing salinity, there was a threshold salinity below which changes in salinity did not result in a detectable decrease in species richness. The number of species per 12 samples was statistically indistinguishable across EC categories < 0.49 mS·cm^{-1}, and the $S_{obs}(12)$ value did not decline by a statistically significant amount until the 1.0–1.49 mS·cm^{-1} category. However, the 0.30–0.499 and 0.50–0.999 mS·cm^{-1} categories had insignificantly lower $S_{obs}(12)$ values than the lower salinity categories, and the possibility of a type II error has to be acknowledged. Both the number of species per 12 samples and $S_{obs}(12)$ values were slightly (but insignificantly) lower in the <0.050 mS·cm^{-1} category

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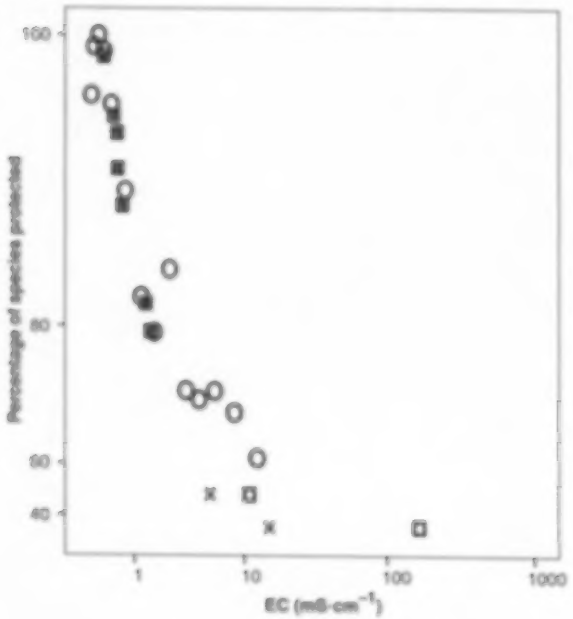
Fig. 6. $S_{obs}(12)$ (the number of species observed in 12 samples) percentage (circles) for each of the electrical conductivity (EC) categories (the midpoint of the categories is plotted) compared with species sensitivity distribution (SSD) with safety factors calculated by a quadratic function (eq. 1; crosses) and exponential function (eq. 2; squares). Note the nonlinear scales of the *x* and *y* axes.



than those in the categories from 0.05–0.9 to 0.2–0.29 mS·cm^{-1}. Furthermore, as EC increased from <0.050 to 0.20–0.29 mS·cm^{-1}, more individuals were collected in 12 samples. These patterns might be due to some stimulatory effect of salinity (see Kefford and Nugegoda 2005; Kefford et al. 2006). Whatever the case and even considering the possibility of type II errors, it would appear that there is no loss of riverine macroinvertebrate species richness in Victoria from the edge habitat associated with increasing salinity below ~0.30–0.50 mS·cm^{-1}. This does not preclude relationships between salinity and macroinvertebrate community structure, loss in the species richness of other organisms, or ecosystem functions at lower salinities.

The exponential and the matching quadratic safety factors imply that species whose dominant life stage has higher 72 h LC_{50} values tend to also be relatively more tolerant of other effects of salinity than species whose dominant life stage has lower LC_{50} values. Other effects of salinity potentially include sublethal (Kefford and Nugegoda 2005; Kefford et al. 2006), chronic lethal, and indirect effects and lower salinity tolerance of early life stages such as eggs and hatchlings (Kefford et al. 2004*b*). Though not specifically demonstrated with salinity, there is also the possibility of intergenerational effects from toxicants (Rose et al. 2004; Zalizniak and Nugegoda 2006). There is insufficient data to fully test whether these other effects can explain the exponential and the matching quadratic safety factors. However, anecdotal comparisons support such an explanation. The older life stages of freshwater macrocrustaceans tend to have higher 72 h LC_{50} values for salinity compared with insects and non-arthropods (Kefford et al. 2003*a*). Kefford et al. (2004*b*) found that the difference in the salinity tolerance between the crustacean hatchlings and their older life stages was less than that of insects and gastropods. Likewise, salinities that reduced growth of the salt-tolerant damselfly *Ischnura heterosticta* (LC_{50} value ~50 mS·cm^{-1}, Kefford et al. 2003*a*) also induced chronic mortality (Kefford et al. 2006).

Effects of stressors and toxicants on individual life-history traits, such as survival, growth, reproduction, etc., do not necessary directly translate into population-level effects, because populations can suffer some reductions in these traits and be unaffected (see Calow et al. 1997; Forbes and Calow 2002*c*). How individual responses affect a population depend on many factors, including the organisms' life history. Furthermore, population-level effects may not necessary translate into community-level responses, such as changes in species richness. The fact that a single safety factor (either exponential- or quadratic-based) applied to only acute survival data matched a community response suggests that across many species of Victorian riverine macroinvertebrates, there might be a general rule of thumb that approximately links the individual response of survival to the community response of species richness. SSDs are, however, often constructed using organisms from more varied taxa (e.g., algae, microinvertebrates, macroinvertebrates, and fish) and habitats (Warne 2001), and thus the variation in life histories is likely to be greater in such SSDs than in the SSD we used. It remains to be shown whether a match among SSDs comprising a wide range of organisms can match the loss of species in nature.

In using any data on the distribution of species in nature with respect to salinity (or other toxicants or stressors), it is necessary to make the assumption that any relationship between species richness is not caused by some other factor(s) that co-vary with salinity (Kefford 1998*b*, 2000). While there are likely aspects of water quality, instream and riparian habitat, climate, or land use that are correlated with salinity, we suggest that this is a reasonable assumption to make. Firstly, if this assumption were not made, it would be practically impossible to test the predictions of any SSD, and thus the use of SSDs requires the assumption that they do protect the percentage of species that they report to protect. Secondly, for factors such as the dissolved oxygen saturation (see Williams 1998) and concentrations of major individual ions (i.e., Na^+, Ca^{2+}, Mg^{2+}, K^+, Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-}) that are causally linked with salinity, there seems to be little point in separating their individual effects from salinity. Saline waters will always have lower dissolved oxygen saturation and higher concentrations of individual ions than fresh waters. Thirdly, for other factors that, while not causally linked with salinity, tend to be correlated with salinity, it makes sense to consider the total effects of rises in salinity and changes in associated confounding variables when managing effects of rising salinity on freshwater biodiversity. Therefore comparing the salinity SSD and loss of species richness caused by salinity and possibly some other unknown factors seems worthwhile. Fourthly, salinity tolerance experiments conducted at different pHs (L. Zalizniak, RMIT University, P.O. Box 71, Bundoora, 3083, Australia, personal

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communication) or with the three most common variations in ionic proportions of saline waters of southeast Australia (Radke et al. 2002; Zalizniak et al. 2006) showed surprisingly little variations in salinity tolerance, especially for 72 h and 96 h LC_{50} values.

Finally, it seems unlikely that if salinity were confounded with other factor(s) that caused the reduction in species richness across the EC categories, this reduction could be described by a simple mathematical function applied to an SSD based entirely on laboratory salinity tolerance data. Furthermore, with data available for other effects, such as sublethal, early life stage, and chronic, for more species it will be possible to determine whether the exponential and the matching quadratic safety factors have any biological bases. If there is a biological base in these safety factors, then it would be extremely unlikely that factors confounded with salinity caused the reduction in species richness. Therefore, the assumption that some unknown factor(s) cause the reduction in species richness with increasing EC category can be tested.

It is also possible that the decline in species richness is due to rarity of saline rivers in Victoria. Small habitat patches, or islands, generally support fewer species than larger patches (Begon et al. 1990). The fact that saline rivers in Victoria are relatively small habitat patches relative to freshwater rivers may explain why there are fewer species in saline rivers. If salinity increases gradually, adaptations may increase the salinity tolerance of macroinvertebrates and thus lead to less effects of salinity on macroinvertebrate species richness than the current study indicates. Comparisons with the loss of species richness with increasing salinity between Victoria and other places where salinization has a longer history, such as southwestern Australia (see Kay et al. 2001), may be able to assess the degree to which adaptation can alter the salinity – species richness relationship observed here.

We have confirmed the central prediction of the SSD concept with salinity and freshwater macroinvertebrates. It does not necessarily follow that this confirmation applies for more traditional toxicants (metals, pesticides, hydrocarbons, etc.). Unlike salinity, numerous water quality factors influence the bioavailability of many traditional toxicants (Hall and Anderson 1995; Paquin et al. 2000), and it may therefore be more problematic to directly compare laboratory toxicological results to the distribution of organisms in the environment. However, if the central prediction of the SSD concept had not been confirmed with salinity, it would seem unlikely that this prediction would apply for other toxicants. Therefore, the fact that the central prediction of the SSD concept has been confirmed with salinity in this paper is encouraging for the use of SSDs for other toxicants, perhaps with some correction for bioavailability. Liess and Von der Ohe (2005) recently studied macroinvertebrate species in natural streams exposed to pesticides. They showed that species that were lethally sensitive to organics and (or) metals in single species acute (24–48 h) laboratory tests (Von der Ohe and Liess 2004) and with life-cycle traits known to delay recovery following toxicant-induced disturbance were more affected by diffuse source pesticide exposure than other species. They have thus shown that it is possible to validate, with field data, laboratory sensitivity data for traditional toxicants. More studies validating laboratory sensitivity data and SSD for a range of toxicants and other stressors should be conducted.

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Estimation of seabird bycatch for North Pacific longline vessels using design- and model-based methods

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Abstract: Bycatch estimation for sensitive species is becoming increasingly important with the shift toward an ecosystem-based approach to fisheries management. Incidental mortalities for various seabird species occur on longline vessels throughout the world, including those in the North Pacific groundfish fleet. We present an approach to seabird bycatch estimation for North Pacific longline vessels using observer-collected data. Observers collect enormous amounts of data through a complex sampling design, but some information deficiencies preclude bycatch estimation using only probability sampling. Our approach combines probability sampling with model-dependent techniques to overcome these information deficiencies. The resulting bycatch estimator reflects the observer sampling design as closely as possible and minimizes reliance on untested model assumptions. We apply our estimator to black-footed albatross (*Phoebastria nigripes*) bycatch as an example and compare yearly estimates to those previously published. We also suggest changes in data collection that would further reduce dependence on model assumptions.

Résumé : L'estimation des prises accessoires d'espèces vulnérables devient de plus en plus importante à mesure que l'on adopte une approche plus centrée sur l'écosystème dans la gestion des pêches. Des mortalités fortuites de diverses espèces d'oiseaux marins sont causées par les navires de pêche à la palangre partout dans le monde, y compris par les navires de la flotte de pêche des poissons de fond du Pacifique Nord. Nous présentons une méthode pour estimer les captures accessoires d'oiseaux marins par les navires de pêche à la palangre du Pacifique Nord basée sur des données récoltées par des observateurs. Les observateurs récoltent des quantités énormes de données selon un plan d'échantillonnage complexe, mais des failles dans l'information empêchent l'estimation des prises accessoires à l'aide d'un seul échantillonnage de probabilité. Notre méthode combine l'échantillonnage de probabilité avec des techniques reliées au modèle afin de contrer les manques d'information. L'estimateur des prises accessoires obtenu représente autant que possible le plan d'échantillonnage utilisé par les observateurs et il minimise la dépendance vis-à-vis des suppositions non vérifiées du modèle. Comme exemple, nous utilisons notre modèle pour étudier les prises accessoires d'albatros à pieds noirs (*Phoebastria nigripes*) et nous comparons les estimations annuelles obtenues à celles déjà publiées. Nous suggérons aussi des modifications dans la cueillette des données pour réduire davantage la dépendance vis-à-vis des présuppositions du modèle.

[Traduit par la Rédaction]

Introduction

The impact of bycatch on marine ecosystems has been the subject of much research interest (e.g., Alverson et al. 1994; Crowder and Murawski 1998; Hall et al. 2000), and with the movement toward ecosystem-based management (e.g., Fluharty and Cyr 2001), there is increasing concern for bycatch and other indirect effects of fishing by the North Pacific groundfish fisheries (e.g., Whiterell and Pautzke 1997; Witherell et al. 2000; Stehn et al. 2001). Bycatch of seabirds due to longline gear, specifically, has received much attention (Brothers et al. 1999), and there has been an effort to minimize this impact in the North Pacific Ocean (Melvin et al. 2001).

Estimation of bycatch, including that of seabirds, requires statistically sound approaches. Design-based inference is a well-established approach to estimation that relies on the random selection procedures used to collect data (Cochran 1977; Särndal et al. 1992). A design-based estimator is a function of the probabilities that elements are included in the observed sample using the random selection procedures. Regardless of the process that generates the values in the population being sampled, design-based estimators can provide unbiased population parameter estimates and corresponding measures of variance. On the other hand, model-based inference in finite population sampling is a more recently developed approach that assumes a superpopulation model generates the values realized for each element in the finite set being sampled (Cassel et al. 1977; Valliant et al. 2000). Model-based estimation is achieved by predicting the values for the unsampled elements in the universe

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with functions of the observed elements. Jolly and Hampton (1990) and Smith (1990) provide insightful examinations of design- and model-based estimation in the context of marine sampling surveys.

Bycatch estimates for overharvested, rare, or endangered species can result in contentious management decisions (e.g., reduced fishing quotas and earlier fishery closures). Results that rely on fewer untested model assumptions are less prone to debate and litigation. Design-based bycatch estimators that rely on no parametric model assumptions would therefore appear to be of interest, but these types of estimators for seabird bycatch are not currently available from data collected by observers in the North Pacific Groundfish Observer Program (NPGOP) because of the complexity of and information deficiencies inherent to the sampling design (see NPGOP sampling design for details).

Progress has been made in changing the NPGOP design to allow completely design-based estimation (e.g., Vølstad et al. 1997; MRAG 2000, 2003), but estimators valid for use with existing data are lacking. We present an estimator for seabird bycatch in the longline sector of the North Pacific groundfish fleet that reflects the randomization of the NPGOP sampling design, but also incorporates some models to deal with information deficiencies in present data records. We apply the estimator to black-footed albatross (*Phoebastria nigripes*) bycatch as an example and compare yearly results to those previously reported.

Materials and methods

NPGOP sampling design

The NPGOP observers collect a variety of data on both targeted and untargeted species occurring in groundfish vessel catches through a stratified, three-phase sampling design (see Särndal et al. 1992, pp. 100–104 and 343–350). Under current regulations, large longline vessels ($\geq$38.1 m in length) generally have observers on board during all fishing activities, medium vessels (<38.1 m and $\geq$18.3 m in length) generally have observers on board 30% of the days spent fishing in each quarter, but small vessels (<18.3 m in length) do not require any observer coverage (AFSC 2004). In meeting their coverage requirement, operators of medium vessels determine which fishing trips will be observed (our use of the term "fishing trip" indicates the duration from the time a vessel departs with an empty hold to the time it unloads any catch). Because each medium vessel must comply with coverage requirements within each quarter, the sampling processes for each vessel each quarter (vessel-quarter) are independent. Vessel-quarters are therefore viewed as strata from a finite sampling perspective. A slight caveat is that vessels that normally have 30% observer coverage requirements may rarely fish under a Community Development Quota where they must have 100% observer coverage. We therefore treat the hauls made by a medium vessel under a Community Development Quota as a separate vessel-quarter with 100% observer coverage.

Within each stratum, there are three nested sampling phases: sampling of trips within a vessel-quarter, sampling of hauls within trips, and sampling of hooks within hauls. When observed fishing trips are representative of all trips during a vessel-quarter, we may assume simple random sampling (SRS) of trips (e.g., Cochran 1977, pp. 18–20). However, trips must be defined by the data user because the NPGOP archives do not distinguish unique trips for each vessel. One criterion to define trips is when observers embark and disembark a particular vessel, but when the vessel performs multiple trips with the observer on board, the separate trips are not distinguishable. In addition, an observer may reboard a vessel at a later date, and the only way to determine this from the NPGOP archives is a break in the dates when hauls are observed. Because the amount of time between consecutive trips with the same observer is unknown, we assume here for simplicity a break of two days between hauls to distinguish trips.

During a fishing trip, observers select hauls for data collection according to an elaborate randomization process. An SRS of hauls within an observed fishing trip is assumed as an approximation to the haul-sampling process (MRAG 2003).

Within the selected hauls, observers typically randomly sample portions of the haul (tally period) to count numbers caught for different species (AFSC 2004). Data are not recorded for different sections of a sampled haul, although the sections are the units that are sampled. Only the numbers of deployed hooks, sampled hooks, and observed seabird mortalities are recorded. For each haul, we assume that the sampled hooks are representative of the unsampled hooks.

Review of other seabird bycatch estimation approaches

Stehn et al. (2001) made the important first effort to provide estimates of seabird bycatch incurred by longliners in the North Pacific groundfish fleet. These authors provided yearly and regional seabird bycatch estimates for 1993–1997. They used a stratified ratio estimator (Cochran 1977, pp. 164–169) by post-stratifying observer-collected data on all seabird bycatch and fish catch weight by 4-week periods (13 per year) and region (Bering Sea – Aleutian Islands (BSAI) and Gulf of Alaska (GOA)). The species-specific, seabird bycatch estimates for a region and period were computed as the sum of (*i*) the species-specific number caught during observed portions of hauls, (*ii*) the number of observed but unidentified birds multiplied by the proportion (P) of the species among all identified birds during observed portions of hauls, and (*iii*) the product of the total weight (kg) of all fish caught less the weight of fish caught during observed portions of hauls (W), the ratio of total seabird bycatch per kilogram of caught fish in observed portions of hauls (R), and P. The first and third components of the summation were computed using data from each post-stratum, but they calculated the second component using moving average techniques. For their variance estimates, Stehn et al. (2001) assumed that P and R were independent and W was measured without error. The estimates of bycatch and variance for a region over an entire year were the sums of the corresponding estimates for the 4-week periods within the year.

Following the initial work by Stehn et al. (2001), NPFMC (2001) also made seabird bycatch estimates for a larger time span using a stratified ratio estimator, but with some key changes in data usage. Rather than making the assumption that seabird bycatch is related to fish catch, the extrapolation is based on the total numbers of observed and unobserved hooks deployed by the longline vessels. Also, the observed species-specific bycatch is extrapolated rather than calculated as the product of the observed total seabird bycatch and P. Lastly, the authors limit the extrapolation to fishing effort made by vessels that had some

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observer coverage in a given post-stratum. The time span for which estimates based on this technique are available has since been expanded to 1993–2002 by NPFMC (2003).

The estimates provided by Stehn et al. (2001) and NPFMC (2003) have provided critical information to the management of North Pacific groundfish fisheries, but there are several assumptions implicit in their estimation approaches that could potentially result in bias of the bycatch estimators and (or) the corresponding variance estimators. As acknowledged by NPFMC (2003), differences in species-specific bycatch rates between vessels or even between medium and large size classes will result in biased estimation of total bycatch for the species because of the unequal sampling rates for the two size classes. A much higher proportion of the total fishing effort is sampled for the large vessels because of the 100% observer coverage requirement than the medium vessels with only a 30% observer coverage requirement. Similarly, the combination of heterogeneity in capture rates and levels of sampling effort across longline hauls in the same region and 4-week period will also lead to estimation bias. Another concern arises in estimating the number of a species among the unidentified birds using P (Stehn et al. 2001). This implies that the probability of identification is equal for all species, and if some species deteriorate faster than others or if some species are harder to tell apart than others, then this assumption would not hold and estimation bias would result. Also, W and the numbers of total hooks deployed are assumed known, but these are estimates that rely on both observer- and industry-supplied catch estimates (see Battaile et al. 2005 for more details). Observer-based catch estimates are likely unbiased owing to randomized sampling, but any departure of industry-based estimates from true total catches would cause bias in the seabird bycatch estimates as well.

There will also be bias in the estimation of sampling variance using the methods of Stehn et al. (2001) and NPFMC (2003). There is unpropogated uncertainty in the measures of unobserved effort (catch weight or deployed hooks) to which the estimated bycatch ratio is extrapolated. By itself, the treatment of the unobserved effort as known would cause the variance estimate to be negatively biased, but covariance among the different component estimates could be either negative or positive and provide an unpredictable contribution to the bias of the variance estimates. Finally, the stratified three-phase design used by the NPGOP produces covariance among the bycatch estimates computed for each of the 4-week, regional post-strata. The covariance arises because observers sample hauls randomly within trips rather than within the 4-week, regional post-strata, and a given trip can contain hauls that occur in multiple post-strata. Their stratified ratio estimator and corresponding variance estimator are appropriate when the strata are defined a priori and randomization procedures are independent across strata. In contrast with post-stratification, independent sampling within predefined strata results in no covariance among estimates for different strata (cf. Cochran 1977, pp. 91–93 and pp. 134–135).

A new seabird bycatch estimator

The seabird bycatch estimator we present for yearly bycatch is a function of nested estimators at the various phases within each vessel-quarter stratum. As such, we start our description of the estimator at the most nested phase and work up (i.e., sampling within hauls, then sampling of hauls within trips, then trips within vessel-quarters), so that subsequent estimators build on those previously described (See List of symbols for all symbol definitions).

Hauls and trips

Under a model where the capture of a given species on a hook in a haul is independent across all hooks in the haul and the capture probability (or rate per hook) is constant throughout the haul, the number of fish counted in the sample from the kth haul is a binomial random variable with mean $E(n_k) = h_k p_k$ and variance $\mathrm{Var}(n_k) = h_k p_k(1 - p_k)$. The so-called best linear unbiased predictor for the species-specific bycatch in the haul under this model is given in eq. 1.1, and the variance of this bycatch predictor is estimated with eq. 1.2 (Table 1). The variance estimator is unbiased for the prediction error variance when the model is correct (Valliant et al. 2000). However, if seabird mortalities are clustered along the string of hooks that comprises the haul, the capture probability will not be constant throughout the haul and this clustering will cause the variance estimator (eq. 1.2) to be negatively biased. The bycatch estimator (eq. 1.1) remains unbiased because of the randomized sampling procedures.

We describe the estimators for yearly bycatch below, but it will become evident that variance of the yearly estimator is a function of sampling trips within vessel-quarters and hauls within trips, as well as sampling within hauls. The total sampling variance of yearly estimates will be dominated by variance due to sampling hauls within trips and trips within vessel-quarters (see Särndal et al. 1992, pp. 139–140). Any bias in variance estimation due to clustering of seabird mortalities within hauls will be negligible because capture rates are allowed to be heterogeneous across hauls. The allowance for different capture probabilities for each longline haul relaxes the assumption made by Stehn et al. (2001) and NPFMC (2003) that the probability of seabird bycatch is equal among different trips or vessels fishing in the same region during a given period.

Under the simple random sampling approximation for the randomization procedure used to select which hauls to sample, we estimate the bycatch occuring in the tth trip with eq. 1.3 and the variance of the bycatch estimator with eq. 1.4. Notice that these estimators for the within-trip phase of sampling are functions of the estimators for the within-haul bycatch and corresponding variance (eqs. 1.1 and 1.2). Furthermore, the estimator and variance estimator for trip-specific bycatch is identical to those appropriate for two-stage simple random sampling, where hooks are randomly sampled at the second stage (e.g. Cochran 1977, p. 303), because the model-based estimators (eqs. 1.1 and 1.2) are the same as the design-based estimators under simple random sampling of hooks.

Simple random sampling is used as an approximation to the random haul-selection procedure because the probabilities that different hauls enter the sample are complex and their calculation has never been performed or reported. The probabilities of sample inclusion for each haul are approximately equal within a given trip for experienced observers. In contrast, estimation approaches used by Stehn et al. (2001) and NPFMC (2003) use simple random sampling approximations for all hauls within regions and periods, regardless of fishing trips or vessel size class. These approaches implicitly assume that each haul in the region

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Table 1. Haul- and trip-specific bycatch estimators and corresponding variance estimators.

(1.1)	$\widehat{N}_k = n_k + (H_k - h_k)\widehat{p}_k = H_k \widehat{p}_k$ where $\widehat{p}_k = n_k / h_k$
(1.2)	$\widehat{\mathrm{Var}}(\widehat{N}_k) = H_k \left(\frac{H_k}{h_k} - 1\right) \frac{h_k \widehat{p}_k (1-\widehat{p}_k)}{h_k - 1}$
(1.3)	$\widehat{N}_t = \frac{G_t}{g_t} \sum_{k=1}^{g_t} \widehat{N}_k$
(1.4)	$\widehat{\mathrm{Var}}(\widehat{N}_t) = G_t \left(\frac{G_t}{g_t} - 1\right) \widehat{S}_t^2 + \frac{G_t}{g_t} \sum_{k=1}^{g_t} \widehat{\mathrm{Var}}(\widehat{N}_k)$ where $\widehat{S}_t^2 = \frac{\sum_{k=1}^{g_t} (\widehat{N}_k - \widehat{\bar{N}}_t)^2}{g_t - 1}$ and $\widehat{\bar{N}}_t = \frac{\widehat{N}_t}{G_t}$
(1.5)	$\widehat{N}_{mt} = \frac{G_{ft}}{g_t} \sum_{k=1}^{g_{mt}} \widehat{N}_k$
(1.6)	$\widehat{\mathrm{Var}}(\widehat{N}_{mt}) = G_t \left(\frac{G_{ft}}{g_t} - 1\right) \left[\frac{\sum_{k=1}^{g_{mt}} \widehat{N}_k^2}{g_{mt} - 1} - \frac{\left(\sum_{k=1}^{g_{mt}} \widehat{N}_k\right)^2}{g_{mt}(g_{mt} - 1)} \right] + \frac{G_{ft}}{g_t} \sum_{k=1}^{g_{mt}} \widehat{\mathrm{Var}}(\widehat{N}_k)$

and period is equally likely to enter the set of sampled hauls, which is not true when observers sample hauls differently for each fishing trips. Potential causes of differences in sampling effort across trips include differences in stamina among observers and differences in weather conditions among trips. If selection probabilities are different between trips, the approaches used by Stehn et al. (2001) and NPFMC (2003) will provide biased estimates of indeterminant magnitude and direction.

Many investigators desire to estimate bycatch for particular regions and (or) periods in the North Pacific for which sampling effort is not explicitly controlled in the sample design. That is, hauls within a given trip are sampled without regard to where or when (within a given quarter) they are fishing, and vessels perform fishing activities that can occur within or outside of the region and (or) period of interest to the investigator on any given fishing trip. Design-based estimation for these subsets of hauls is known as subpopulation (Cochran 1977, pp. 35–38) or domain (Särndal et al. 1992, pp. 390–395) estimation. The domain estimator for bycatch incurred in the tth trip for the mth region and (or) period is eq. 1.5; the corresponding variance estimator is eq. 1.6. Notice that there is no minimum number of hauls in the region and (or) period necessary to make unbiased bycatch or corresponding variance estimates. Furthermore, because the domain estimator accounts for variance of sample sizes within the domain, it will generally have higher variance than an estimator corresponding to a sample design where SRS is used to collect data within the domain. When the domain of interest contains all hauls made during a given trip, the estimator and variance estimator simplify to eqs. 1.3 and 1.4, respectively.

A model-based approach for trips with too few hauls sampled

Infrequently, there are trips where an observer is present on the vessel, but he or she samples less than two hauls (when two or more are made), or upon debriefing, NPGOP personnel determine that data are not collected appropriately by the observer. For these undersampled trips, the number of hauls made and the location and number of hooks deployed for each haul are known.

Table 2. Model-based estimators for trips where samples are insufficient for design-based estimation.

(2.1)	$\widehat{N}^*_{mt} = H_{mt} \widehat{p}_m$ where $H_{mt} = \sum_{k=1}^{g_{mt}} H_k$ and $\widehat{p}_m = \frac{\sum_{v=1}^{V} \sum_{t=1}^{r_v} \sum_{k=1}^{g_{mt}} n_k}{\sum_{v=1}^{V} \sum_{t=1}^{r_v} \sum_{k=1}^{g_{mt}} h_k} = \frac{n_m}{h_m}$
(2.2)	$\widehat{\mathrm{Var}}(\widehat{N}^*_{mt}) = H_{mt} \widehat{p}_m (1 - \widehat{p}_m) \widehat{\tau}_m \left(1 + \frac{H_{mt}}{h_m}\right)$
(2.3)	$\widehat{\tau}_m = \frac{1}{g_m - 1} \sum_{v=1}^{V} \sum_{t=1}^{r_v} \sum_{k=1}^{g_{mt}} \frac{(n_k - h_k \widehat{p}_m)^2}{h_k \widehat{p}_m (1 - \widehat{p}_m)}$ where $g_m = \sum_{v=1}^{V} \sum_{t=1}^{r_v} R_{mt}$
(2.4)	$\widehat{\mathrm{Cov}}(\widehat{N}^*_{mt}, \widehat{N}^*_{mu}) = H_{mt} H_{mu} \frac{\widehat{p}_m (1 - \widehat{p}_m) \widehat{\tau}_m}{h_m}$

We employ a hierarchical model for bycatch probabilities to make bycatch estimates for trips with too few sampled hauls. We assume for an unsampled haul that the expected bycatch in a haul conditional on the haul-specific bycatch probability is $E(N_k|p_k) = H_k p_k$ and the conditional variance is $\mathrm{Var}(N_k|p_k) = H_k p_k (1 - p_k)$. Over all hauls in the mth region and (or) period, the mean capture rate is $E(p_k) = p_m$ and $\mathrm{Var}(p_k) = \sigma^2_m$. Thus, the marginal expected bycatch and variance are $E(N_k) = H_k p_m$ and $\mathrm{Var}(N_k) = N_k p_m (1 - p_m) \tau_m$. This hierarchical approach allows variance to depart from a simple binomial model through the variablity in capture rates across hauls. The corresponding estimator for the bycatch incurred in the mth region and (or) period during the tth insufficiently sampled trip is eq. 2.1; the variance estimator is eq. 2.2 (Table 2). The dispersion estimator (eq. 2.3) is asymptotically unbiased (McCullagh and Nelder 1989, pp. 124–128).

A further variation on this approach is to make model-based estimates using eqs. 2.1 and 2.2 for smaller regions and (or)

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Table 3. Bycatch estimator, corresponding variance estimator, and variance estimator for total deployed hooks within vessel-quarters and over multiple vessel-quarters.

(3.1) $$\hat{N}_v = \frac{c_v}{r_v}\sum_{i=1}^{r_v}\hat{N}_i$$

(3.2) $$\widehat{\mathrm{Var}}(\hat{N}_v) = C_v\left(\frac{c_v}{r_v}-1\right)\frac{\sum_{i=1}^{r_v}\hat{N}_i^2}{r_v} + \frac{c_v}{r_v}\left[\sum_{i=1}^{r_v}\widehat{\mathrm{Var}}(\hat{N}_i) + \frac{c_v-1}{r_v-1}\sum_{i=1}^{r_v}\sum_{\substack{a=1\\ a\neq i}}^{r_v}\widehat{\mathrm{Cov}}(\hat{N}_i,\hat{N}_a)\right]$$

(3.3) where $$\widehat{S}^2_{N,v} = \frac{\sum_{i=1}^{r_v}(\hat{N}_i-\hat{N}_v)^2}{r_v-1}$$

and $$\hat{N}_v = \frac{\hat{N}_v}{r_v}$$

(3.4) $$\hat{N}_v = \xi_v\sum_{i=1}^{c_v}\hat{N}_i$$

(3.5) $$\widehat{\mathrm{Var}}(\hat{N}_v) = \xi_v^2\left[\hat{\sigma}_v^2\hat{N}_v^2 + \sum_{i=1}^{c_v}\widehat{\mathrm{Var}}(\hat{N}_i) + \sum_{i=1}^{c_v}\sum_{\substack{a=1\\ a\neq i}}^{c_v}\widehat{\mathrm{Cov}}(\hat{N}_i,\hat{N}_a)\right] + \xi_v\left[(\xi_v-1)c_v - \frac{\hat{\sigma}_v^2}{c_v}\right]\widehat{S}^2_{N,v}$$

(3.6) where $$\widehat{S}^2_{N,v} = \overline{S}_{N,v} - \frac{1}{c_v}\left[\sum_{i=1}^{c_v}\widehat{\mathrm{Var}}(\hat{N}_i) - \frac{1}{c_v-1}\sum_{i=1}^{c_v}\sum_{\substack{a=1\\ a\neq i}}^{c_v}\widehat{\mathrm{Cov}}(\hat{N}_i,\hat{N}_a)\right]$$

and $$\hat{\sigma}_v^2 = \frac{\sum_{i=1}^{v_M}(c_{vi}-\bar{c}_M)^2}{v_M-1}$$

and $$\bar{c}_M = \frac{\sum_{i=1}^{v_M}c_{vi}}{v_M}$$

(3.7) $$\hat{N} = \sum_{v=1}^{V}\hat{N}_v$$

(3.8) $$\widehat{\mathrm{Var}}(\hat{N}) = \sum_{v=1}^{V}\widehat{\mathrm{Var}}(\hat{N}_v) + \sum_{v=1}^{V}\sum_{\substack{u=1\\ u\neq v}}^{V}\xi_v\xi_u\sum_{i=1}^{c_v}\sum_{a=1}^{c_u}\widehat{\mathrm{Cov}}(\hat{N}_{vi},\hat{N}_{ua})$$

(3.9) $$\widehat{\mathrm{Var}}(\hat{N}_U) = \frac{v_U}{v_U-1}\left[\sum_{v=1}^{v_U}\left(\hat{N}_v-\hat{\bar{N}}_U\right)^2 + \sum_{v=1}^{v_U}\sum_{\substack{u=1\\ u\neq v}}^{v_U}\widehat{\mathrm{Cov}}(\hat{N}_v,\hat{N}_u)\right]$$

where $$\hat{N}_U = V_U\hat{\bar{N}}_U = \sum_{v=1}^{v_U}\hat{N}_v$$

(3.10) $$\widehat{\mathrm{Var}}(\hat{H}_v) = \xi_v^2\hat{\sigma}_v^2\hat{H}_v^2 + \xi_v\left[(\xi_v-1)c_v - \frac{\hat{\sigma}_v^2}{c_v}\right]\widehat{S}^2_{H,v}$$

periods and then sum the estimates within the larger region and (or) period of interest. We use this approach in computing yearly black-footed albatross bycatch estimates for BSAI and GOA; for an undersampled trip, we make estimates for each of several subregional management areas (National Marine Fisheries Service statistical areas) within each of the larger regions and then sum the subregional estimates and variance estimates for the trip. Generally, all of the hauls made during an undersampled trip occur within only one of the smaller management areas.

The proportion of fishing effort for a yearly set of vessel-quarters that occurs in these undersampled trips is quite small, and the proportion of total bycatch in these undersampled trips will correspondingly be small. Nevertheless, the use of a hierarchical model for this small subset of overall effort is more realistic and lets us avoid the more restrictive and unrealistic assumption of constant capture rates for the region and (or) period.

Vessel-quarters

The selection of (observer deployment on) fishing trips is the least nested phase of sampling within the vessel-quarter. Given a total number of trips and the SRS assumption for trip deployment, total bycatch for a vessel-quarter and the corresponding variance are estimated with eqs. 3.1 and 3.2 (Table 3).

For vessel-quarters with 100% observer coverage, all trips are sampled ($C_v = c_v$) and the total bycatch is the sum of the trip-specific bycatch estimates. When both the ith and ath trips within a vessel-quarter are undersampled and eq. 2.1 is used to estimate seabird bycatch, there will be non-zero covariance of these estimates, which is estimated using eq. 2.4. When one or fewer of the trips in the double summation are model-based, there will be no covariance of the trip-specific estimates with respect to the models used for estimation. As with the bycatch estimator, sampling of all trips for vessel-quarters with 100% observer coverage results in a simplification of eq. 3.2;

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the first term vanishes and the variance estimator becomes the sum of the trip-specific variance estimates and any covariance estimates for undersampled trips. When bycatch for the mth region and (or) period is of interest, the appropriate estimator of the total bycatch and corresponding variance estimator for a vessel-quarter have the same form as eqs. 3.1 and 3.2, respectively, but the region- and (or) period-specific bycatch estimators for trips are used (eqs. 1.5 and 1.6).

Unfortunately, the total number of trips made within each vessel-quarter subject to 30% observer coverage is unknown. However, we do know that medium longline vessels are regulated to have at least 30% of their fishing days covered by observers. Since it is not in the financial interest of the vessels to have observers on board fewer or greater than the necessary number of fishing days, an assumption that the number of observed fishing days is 30% of the total number spent fishing appears tenable.

We assume a probability model for the number of observed fishing trips in a vessel-quarter having 30% observer coverage with mean $E(c_v) = C_v/\xi_v$ and variance $\mathrm{Var}(c_v) = \sigma_c^2$, where $\xi_v = 10/3$ is the inverse of the average proportion of sampled trips. Under these assumptions and SRS of trips, eq. 3.4 provides estimates that are unbiased with respect to the joint distribution of c_v and $\sum_{v=1}^{c_v} \widehat{N}_t$. The corresponding variance estimator (eq. 3.5) is conservative (i.e., positively biased) because $\widehat{\sigma}_c^2$ will overestimate the variance of the number of sampled trips unless the mean number of sampled trips is also constant across vessels. As with eq. 3.2, region- and (or) period-specific estimates are made using the corresponding estimators for each trip, and the covariance of trip-specific estimators only occurs when both the tth and uth trips are undersampled.

Although some may question the assumption that observed trips are representative of unobserved trips, this assumption is also implicitly made in most estimators based on observer-collected data when coverage is less than 100%, and it is unavoidable when bycatch estimates for unobserved trips are needed. The earlier estimation approaches of Stehn et al. (2001) and NPFMC (2003) also extrapolate data from observed fishing effort to unobserved fishing effort. However, whereas previous methods assume that fishing effort for different vessels and different vessel size classes are representative of others, eqs. 3.4 and 3.5 account for the independence of sampling across vessel-quarters, and the estimates are weighted according to expected proportions of observed trips for each vessel-quarter. When the assumption of representativeness does not hold, estimation must be limited to observed trips.

Quarterly and yearly bycatch estimation

Because vessel-quarters are strata, the bycatch estimator for any arbitrary set of V vessel-quarters is the sum of the estimators for each vessel-quarter (eq. 3.7). The quarterly and yearly bycatch estimators are just special cases where the set of vessel-quarters is those occurring in the quarter or year, respectively. However, the variance estimator must, in general, account for the covariance of model-based estimates for any undersampled trips (eq. 3.8). The scalars for the covariance terms are $\xi_v = 1$ and $\xi_v = 10/3$ for vessel-quarters with 100% and 30% observer coverage, respectively. The covariance estimator for trip totals is non-zero when both trips are undersampled and has a form identical to eq. 2.4, but when there are no undersampled trips, eq. 3.8 reduces to the familiar stratified sampling variance estimator (Särndal et al. 1992, p. 102). The covariance among trips in different vessel-quarters will only be non-zero for those with undersampled trips where bycatch estimates are made from the same data. For example, if the hierarchical models for bycatch capture probability are quarter-specific, then there will be covariance of bycatch estimates for undersampled trips within the same quarter but not of bycatch estimates for undersampled trips in different quarters. The models on which our yearly black-footed albatross bycatch estimators for undersampled trips are based are all quarter-specific.

The last complication of the NPGOP sampling design to be dealt with is the occurrence of vessel-quarters with 30% observer coverage and a single observed trip (undersampled vessel-quarters). This is similar to the undersampling of trips, which we treated with model-based methods. However, there is always at least one trip sampled in each vessel-quarter, whereas there could be no hauls sampled in an undersampled trip. Therefore, bycatch estimation based only on data collected in a given vessel-quarter is possible using eq. 3.4 whether it is undersampled or not, but variance estimation is not possible using eq. 3.5. We use an alternative, conservative estimator for the variance over all undersampled vessel-quarters (eq. 3.9). This variance estimator parallels the variance estimator for collapsed strata given by Cochran (1977, pp. 138–140), which is useful for stratified sampling when only one element is sampled within some strata. The difference here is that we must account for the covariance between any model-based, trip-specific bycatch estimators for different vessel-quarters. In making our yearly black-footed albatross bycatch estimates, we collapse undersampled vessel-quarters within each quarter.

Bycatch rates

Bycatch rates are important for analyzing changes in bycatch over time (e.g., due to implementation of bycatch deterrents) because changes in amounts of effort are controlled. The yearly bycatch rate (per hook) is a function of all fishing effort of both large and medium vessels. If we measure rates for just the observed trips, incorrect weight is attributed to fishing effort made in vessel-quarters with different observer coverage levels.

To estimate a yearly bycatch rate, we need a yearly estimate of total deployed hooks along with the estimate of yearly total bycatch. For vessel-quarters with 100% observer coverage, the total number of hooks is known because the observer records the number of hooks deployed in both sampled and unsampled hauls. However, there is uncertainty in the number of deployed hooks for medium vessels because of unobserved trips. Under the same assumptions of trip sampling within vessel-quarters that we made for seabird bycatch estimation, we can estimate total numbers of deployed hooks for vessel-quarters with 30% observer coverage using eq. 3.4, except the number of hooks per trip is known rather than estimated (i.e., eqs. 1.3 or 1.5 are not needed). Because the number of hooks deployed in each trip are known, eq. 3.5 reduces to eq. 3.10, where $\widehat{S}_{H,v}^2$ is analogous to eq. 3.3. Also, the collapsed strata variance estimator is used to provide conservative variance estimation of the total hooks estimator over the set of undersampled vessel-quarters (eq. 3.9). Over a set of Q quarters (e.g., within a year), the seabird bycatch

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Table 4. Haul-specific estimates of black-footed albatross (*Phoebastria nigripes*) bycatch ($\widehat{N}_i$), standard errors ($\widehat{\mathrm{SE}}$), and numbers of hooks deployed (H_i) and sampled (h_i) for a trip by a medium size class vessel fishing in the second quarter of 2001 (eight of nine hauls made during the trip were sampled (hence, NA); the trip estimates are in the bottom row).

Haul	H_i	h_i	n_i	$\widehat{N}_i$	$\widehat{\mathrm{SE}}(\widehat{N}_i)$
1	4850	1552	0	0	0
2	4850	1552	0	0	0
3	4584	1530	0	0	0
4	5597	1738	0	0	0
5	4600	1472	0	0	0
6	6272	1766	0	0	0
7	4725	1511	2	6.254	3.646
8	3298	1164	0	0	0
9	4704	0	NA	NA	NA
Trip				7.036	4.523

rate estimator is

$$\widehat{R} = \frac{\sum_{q=1}^{Q}\sum_{v=1}^{V_q}\widehat{N}_v}{\sum_{q=1}^{Q}\sum_{v=1}^{V_q}\widehat{H}_v} = \frac{\widehat{N}}{\widehat{H}}$$

and the corresponding variance estimator (by a delta method approximation) is

$$\widehat{\mathrm{Var}}(\widehat{R}) = \widehat{R}^2\left[\frac{\widehat{\mathrm{Var}}(\widehat{N})}{\widehat{N}^2} + \frac{\widehat{\mathrm{Var}}(\widehat{H})}{\widehat{H}^2} - 2\frac{\widehat{\mathrm{Cov}}(\widehat{N},\widehat{H})}{\widehat{H}\widehat{N}}\right]$$

The covariance estimator for total bycatch and deployed hooks is analogous to eq. 3.8.

Results

Black-footed albatross example

To illustrate how the new bycatch estimator is used, we present an example for black-footed albatross bycatch using observer data collected in 2001. Similar to the description of the estimator, we start our example at the most nested phase of the NPGOP sampling design with data collected by an observer during a trip made by a medium vessel during the second quarter of 2001 (Table 4). The number of hooks deployed and sampled and the number of black-footed albatross observed during the tally periods for each sampled haul are the necessary data for the model-based haul estimates of total bycatch. Of the nine hauls made during the first trip, eight were sampled by the observer. Notice that most of the sampled hauls have no observed black-footed albatross bycatch. This is also the case for most observed trips throughout the North Pacific groundfish fleet. In fact, no black-footed albatross bycatch was observed for any sampled hauls during the fourth quarter of 2001.

Table 5. Estimates of black-footed albatross (*Phoebastria nigripes*) bycatch ($\widehat{N}_t$), standard errors ($\widehat{\mathrm{SE}}$), and numbers of hauls completed (G_t) and sampled (g_t) for two trips on a vessel in the medium size class in the second quarter of 2001 (the first trip is that presented in Table 4; the vessel-quarter estimates are in the bottom row).

Trip	G_t	g_t	$\widehat{N}_t$	$\widehat{\mathrm{SE}}(\widehat{N}_t)$
1	9	8	7.036	4.523
2	12	10	0	0
Vessel-quarter			23.456	28.679

The vessel made two observed trips during the second quarter and both of them were sampled sufficiently so that model-based estimation was not necessary (Table 5). No black-footed albatross bycatch was observed on the second observed trip, which resulted in bycatch (eq. 1.3) and sampling variance (eq. 1.4) estimates of zero. Using the trip-specific bycatch estimates with eqs. 3.4 and 3.5, the bycatch estimate (23.456) and corresponding standard error estimate (28.679) for the vessel-quarter were obtained.

To give an example of model-based estimation for an undersampled trip, we turn to another vessel that performed a fishing trip in the second quarter of 2001 with an observer on board, but where there are no hooks sampled within any of the hauls. The number of sampled hooks and observed black-footed albatross mortalities by management region during the second quarter of 2001 are required to make model-based estimates of region-specific bycatch rates (Table 6). Black-footed albatross bycatch was only observed for hauls made in four National Marine Fisheries Service (NMFS) statistical areas (one in the BSAI and the others in the GOA). Using the number of deployed hooks and the NMFS statistical area where each haul was made during the undersampled trips with the model parameter estimates (Table 6), we estimate bycatch and estimate corresponding variance for the undersampled trips (eqs. 2.1 and 2.2). During the observed but unsampled trip, there were four hauls all made in NMFS statistical area 640, and the total number of deployed hooks was 13 370. The resulting estimate of black-footed albatross bycatch for the trip is $\widehat{N}_t^* = 0.174$ and the standard error estimate is $\widehat{\mathrm{SE}}(\widehat{N}_t^*) = 0.521$.

Over the entire second quarter of 2001, 103 trips were made in 25 vessel-quarters with 100% observer coverage, and 110 observed trips were made in 55 vessel-quarters with 30% observer coverage. Fifteen and seven trips from vessel-quarters with 100% and 30% observer coverage, respectively, were undersampled, which accounted for ~ 1% of all longline hauls in the second quarter of 2001. Black-footed albatross bycatch mortalities were observed in two and four trips from vessel-quarters with 100% and 30% observer coverage, respectively. For the remaining 191 trips that were not undersampled, $\widehat{N}_t =$

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Table 6. Number of hauls (g_m), observed hooks (h_m), black-footed albatross (*Phoebastria nigripes*) mortalities (n_m), estimated bycatch probability ($\widehat{p}_m$), and dispersion parameters ($\widehat{\tau}_m$) for various National Marine Fisheries Service statistical areas (m) during the second quarter of 2001.

m	n_m	h_m	g_m	$\widehat{p}_m$	$\widehat{\tau}_m$
509	0	687 349	256	0	0
512	0	118 109	42	0	0
513	0	144 614	64	0	0
516	0	25 716	5	0	0
517	1	244 474	114	4.09×10^{-6}	1.255
518	0	101 903	56	0	0
519	0	51 087	34	0	0
521	0	3 254 122	873	0	0
523	0	253 500	78	0	0
524	0	541 852	152	0	0
541	0	300 719	168	0	0
542	0	149 671	95	0	0
543	0	234 673	98	0	0
610	0	423 698	241	0	0
620	0	189 439	104	0	0
630	3	481 279	290	6.233×10^{-6}	0.765
640	4	307 590	201	1.3×10^{-5}	1.493
650	1	243 849	158	4.101×10^{-6}	0.788
659	0	291	2	0	0

$\widehat{\mathrm{Var}}\left(\widehat{N}_t\right) = 0$. For the 28 vessel-quarters with 30% observer coverage and more than one trip sampled, we used the variance estimator that incorporates uncertainty in the total number of fishing trips (eq. 3.5) with trip-specific bycatch and variance estimates (Table 7). The bycatch estimates of the 27 undersampled vessel-quarters were used with eq. 3.9 to make a conservative variance estimate over all the undersampled vessel-quarters (114.8).

Quarterly and yearly bycatch estimates are made by summing estimates across vessel-quarters within the respective period (Table 8). For clarity, estimates are summed over all vessel-quarters from vessels within each size class before summing over size classes. The estimated coefficient of variation ($\widehat{\mathrm{CV}}$) decreases as strata are aggregated, so that for a quarter $\widehat{\mathrm{CV}}\left(\widehat{N}_q\right)$ is ~ 80%, but over the entire year $\widehat{\mathrm{CV}}\left(\widehat{N}\right)$ reduces to ~ 40%.

Comparison of yearly black-footed albatross bycatch estimates

We compare our estimates for black-footed albatross bycatch during the years 1993 through 2003 in the BSAI and the GOA with those reported by Stehn et al. (2001) and NPFMC (2003). Over the 5 years that estimates from Stehn et al. (2001) are available, bycatch estimates for the BSAI are similar for all three methods on average, but the corresponding standard error estimates we obtained using eq. 3.8 and those provided by NPFMC (2003) are ~ 150% greater than those provided by Stehn et al. (2001) (Table 9). Furthermore, the estimated coefficients of variation provided by Stehn et al. (2001) are less in each of the 5 years than those obtained using our estimators and those provided by NPFMC (2003). The correlation of our BSAI black-footed albatross bycatch estimates with those provided by Stehn et al. (2001) is 0.686.

Table 7. Number of observed trips (c_v), estimates of black-footed albatross (*Phoebastria nigripes*) bycatch ($\widehat{N}_v$), and standard errors ($\widehat{\mathrm{SE}}$) for vessel-quarters (v) in the medium size class with more than one trip sampled during the second quarter of 2001.

v	c_v	$\widehat{N}_v$	$\widehat{\mathrm{SE}}\left(\widehat{N}_v\right)$
1	3	0	0
2	12	0	0
3	5	0.119	0.454
4	4	0	0
5	5	0	0
6	5	0	0
7	2	23.453	28.679
8	2	0	0
9	2	0	0
10	2	0	0
11	2	0	0
12	2	0.004	0.071
13	2	0	0
14	2	0	0
15	2	9.746	11.906
16	2	0	0
17	2	0	0
18	2	0	0
19	2	0	0
20	3	0	0
21	2	0	0
22	2	10.000	12.780
23	2	0	0
24	2	0	0
25	2	0	0
26	2	0	0
27	6	0	0
28	2	0	0

Over the 10 years estimates are available from NPFMC (2003), eq. 3.7 provided black-footed albatross bycatch estimates for the BSAI ~ 30% greater than those provided by NPFMC (2003), and corresponding standard error estimates provided by eq. 3.8 were ~ 35% greater than those provided by NPFMC (2003). However, our estimated coefficients of variation were less than those provided by NPFMC (2003) in 6 of 9 years with non-zero bycatch estimates. The correlation of our BSAI black-footed albatross bycatch estimates with those provided by NPFMC (2003) was 0.907.

For the GOA, yearly (1993–1997) black-footed albatross bycatch estimates provided by Stehn et al. (2001) are ~ 150% and ~ 200% greater than those provided by eq. 3.7 and NPFMC (2003), respectively (Table 10). In contrast, the estimated coefficients of variation provided by Stehn et al. (2001) are less than those provided by our estimators in each of the 5 years and are ~ 65% and ~ 45% less than those provided by eq. 3.7 and NPFMC (2003). The correlation of our GOA black-footed albatross bycatch estimates with those provided by Stehn et al. (2001) is −0.111.

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Table 8. Quarterly and overall estimates of black-footed albatross (*Phoebastria nigripes*) bycatch by vessel size class (M, medium; L, large) for 2001.

Quarter	$\hat{N}_L$	$\widehat{SE}(\hat{N}_L)$	$\hat{N}_M$	$\widehat{SE}(\hat{N}_M)$	$\hat{N}$	$\widehat{SE}(\hat{N})$
1	0.00	0.00	18.09	18.61	18.09	18.61
2	24.70	12.22	43.32	33.58	68.03	35.74
3	4.69	4.16	12.26	11.11	16.95	11.87
4	0.00	0.00	0.00	0.00	0.00	0.00
Year	29.39	12.90	73.67	39.97	103.07	42.00

Table 9. Yearly estimates of black-footed albatross (*Phoebastria nigripes*) bycatch ($\hat{N}$), standard errors ($\widehat{SE}$), and coefficients of variation ($\widehat{CV}$) in the Bering Sea and Aleutian Islands compared with those presented by Stehn et al. (2001) and NPFMC (2003).

	Eqs. 3.7 and 3.8			Stehn et al.			NPFMC		
Year	$\hat{N}$	$\widehat{SE}(\hat{N})$	$\widehat{CV}(\hat{N})$	$\hat{N}$	$\widehat{SE}(\hat{N})$	$\widehat{CV}(\hat{N})$	$\hat{N}$	$\widehat{SE}(\hat{N})$	$\widehat{CV}(\hat{N})$
1993	33.15	18.61	0.56	11	2.4	0.22	11	5.0	0.45
1994	36.75	13.14	0.36	40	6.1	0.15	37	14.5	0.39
1995	72.59	18.37	0.25	52	6.4	0.12	66	20.5	0.31
1996	19.77	9.67	0.49	23	4.3	0.19	20	14.0	0.70
1997	6.61	3.80	0.58	27	5.4	0.20	9	6.5	0.72
1998	7.87	5.16	0.66	—	—	—	9	6.0	0.67
1999	32.60	21.64	0.66	—	—	—	18	8.0	0.44
2000	34.95	23.37	0.67	—	—	—	16	8.5	0.53
2001	3.89	3.20	0.82	—	—	—	4	4.0	1.00
2002	0.00	0.00	—	—	—	—	0	0.0	—
2003	25.44	21.24	0.84	—	—	—	—	—	—

Over 1993–2002, the GOA bycatch estimates provided by eq. 3.7 were ~ 20% greater than those provided by NPFMC (2003). Similarly, the standard error estimates provided by eq. 3.8 were ~ 55% greater, and our estimated coefficients of variation were greater than those provided by NPFMC (2003) in 9 of 10 years. The correlation of our GOA black-footed albatross bycatch estimates with those provided by NPFMC (2003) was 0.964.

Bycatch rates

We also calculated yearly bycatch rates by region using the bycatch estimates (Tables 9 and 10) and the estimated number of deployed hooks (Table 11). The estimated number of deployed hooks has increased dramatically over 1993–2003 in the BSAI, but there is no apparent trend in catch rates in the BSAI over the same period (Fig. 1). There is much less effort (deployed hooks) in the GOA than in the BSAI, but the bycatch rates in the GOA are much greater than the those in the BSAI.

Discussion

Our estimation of bycatch is based on a combination of probability sampling and model-based prediction within each haul. That is, uncertainty is predominantly design-based, but the uncertainty at the most nested phase (sampling within hauls) is model-based. Of course, we do also use model-based methods for the undersampled trips, but the proportion of effort in quarterly or yearly aggregations of fishing effort attributed to these trips is minute (e.g., ~ 1% of all longline hauls in the second quarter of 2001). In multistage designs (and the NPGOP multiphase design), the proportion of overall uncertainty due to sampling at the finest scale generally becomes negligible as the number of stages increases. Thus, as long as the bycatch rate within each haul is properly modeled, the variance of total bycatch estimation at quarterly or yearly scales will be negligibly affected by improper specification of within-haul variance. On the other hand, if we wished to estimate bycatch within a specific trip or haul, we would require an accurate model of the within-haul variance.

If completely design-based estimation is desirable, changing data-recording protocols for within-haul sampling and tracking the total amount of fishing effort made by medium vessels (i.e., numbers of trips) will be important actions to take. With changes in fishing effort reporting by the Alaska Regional Office, we may be able to use realized observer coverage levels for each vessel-quarter rather than assume 30% and model the number of observed trips in each vessel-quarter. Furthermore, the ability to distinguish observed fishing trips in the NPGOP database would remove the necessity for the investigator to define them.

As seen from the comparison of yearly results, bycatch estimates and variance estimates that recognize the NPGOP sam-

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Table 10. Yearly estimates of black-footed albatross (*Phoebastria nigripes*) bycatch ($\hat{N}$), standard errors ($\widehat{\mathrm{SE}}$), and coefficients of variation ($\widehat{\mathrm{CV}}$) in the Gulf of Alaska compared with those presented by Stehn et al. (2001) and NPFMC (2003).

	Eqs. 3.7 and 3.8			Stehn et al.			NPFMC		
Year	$\hat{N}$	$\widehat{\mathrm{SE}}(\hat{N})$	$\widehat{\mathrm{CV}}(\hat{N})$	$\hat{N}$	$\widehat{\mathrm{SE}}(\hat{N})$	$\widehat{\mathrm{CV}}(\hat{N})$	$\hat{N}$	$\widehat{\mathrm{SE}}(\hat{N})$	$\widehat{\mathrm{CV}}(\hat{N})$
1993	40.86	17.36	0.42	647	93.4	0.14	29	10.5	0.36
1994	12.05	9.60	0.80	803	150.9	0.19	7	4.5	0.64
1995	285.28	54.79	0.19	984	125.9	0.13	236	34.0	0.14
1996	755.31	138.37	0.18	496	82.0	0.17	658	101.0	0.15
1997	139.14	57.62	0.41	123	34.2	0.28	99	34.0	0.34
1998	191.62	85.79	0.45	—	—	—	289	153.5	0.53
1999	279.79	138.79	0.50	—	—	—	183	57.0	0.31
2000	239.27	142.78	0.60	—	—	—	139	43.0	0.31
2001	99.32	41.88	0.42	—	—	—	72	26.0	0.36
2002	54.40	44.84	0.82	—	—	—	33	12.0	0.36
2003	110.04	41.57	0.38	—	—	—	—	—	—

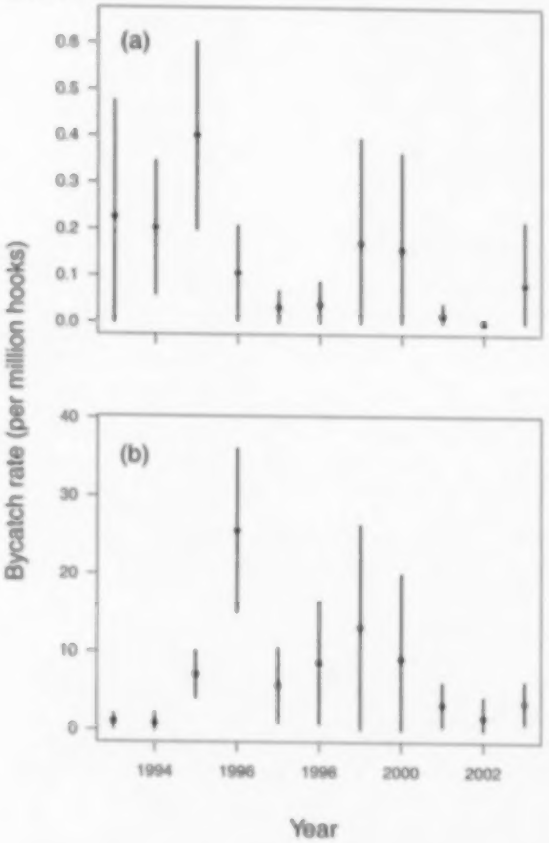
Table 11. Yearly estimates of total deployed hooks ($\times 10^6$, $\hat{H}$) and standard errors ($\widehat{\mathrm{SE}}$) for the Bering Sea – Aleutian Islands (BSAI) and the Gulf of Alaska (GOA).

	BSAI		GOA	
Year	$\hat{H}$	$\widehat{\mathrm{SE}}(\hat{H})$	$\hat{H}$	$\widehat{\mathrm{SE}}(\hat{H})$
1993	146.548	7.336	38.719	3.963
1994	181.111	8.818	14.695	2.764
1995	180.851	7.814	40.173	3.899
1996	186.900	9.109	29.527	2.954
1997	207.982	6.787	24.469	2.688
1998	205.865	7.639	22.164	2.574
1999	189.993	7.556	21.180	2.206
2000	222.488	8.424	26.165	2.625
2001	254.662	8.969	30.486	3.777
2002	248.433	8.683	34.126	3.614
2003	310.098	10.218	31.163	3.104

pling design differ sometimes substantially from those based entirely on models. A major reason for these differences is that previously reported estimates weigh all haul data collected in a given region and period equally rather than according to the different levels of sampling effort during each fishing trip and according to the different observer coverage rates for each size class of vessels. Another factor contributing to the differences in variance estimates is that those provided by Stehn et al. (2001) and NPFMC (2003) are functions of unpropagated variance in estimated catches from unobserved hauls and unpropagated covariances of different component estimators.

The estimates of GOA black-footed albatross bycatch that we obtained using eq. 3.7 are more similar to those provided by NPFMC (2003) than to those provided by Stehn et al. (2001). The reasons for the similarities are not entirely clear, but the fact that NPFMC (2003) excluded fish catches from unobserved vessels may be an important factor. Because the ratios of black-footed albatross bycatch are only extrapolated to catches made by vessels subject to observer coverage and because our estimators only apply to fishing effort subject to observer coverage,

Fig. 1. Estimates of black-footed albatross (*Phoebastria nigripes*) bycatch rates (per 10^6 hooks) in the (*a*) Bering Sea and Aleutian Islands and (*b*) Gulf of Alaska due to fishing effort subject to North Pacific Groundfish Observer Program coverage from 1993 to 2003. Vertical bars represent asymptotic 95% confidence intervals.



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the frames of inference are similar. Stehn et al. (2001) did not limit their estimation to vessels subject to observer coverage, and the resulting estimates are much higher. If a substantial portion of the total fish catch in the GOA is made by small vessels not subject to observer coverage, this would partially explain the difference between bycatch estimates provided by Stehn et al. (2001) and ours, as well as differences between estimates provided by NPFMC (2003) and Stehn et al. (2001).

Beyond the estimation technicalities, black-footed albatross bycatch due to fishing in Alaska seems to be a small contributor to global bycatch of this species. Relative to yearly bycatch estimates for other domestic fishing in the central North Pacific provided by Lewison and Crowder (2003), bycatch in Alaska groundfish fisheries appear to be ~ 10% of the total domestic annual bycatch. However, there may be other types of interactions with the groundfish fleet, such as facultative feeding on discards from these vessels, which has been reported for other seabirds (Garthe et al. 1996; Oro 1996; Votier et al. 2004).

Finally, an area of research that may play an important role in ecosystem-based management of the North Pacific is multispecies population modeling. Obviously, it is necessary to consider fishing-induced mortality of seabirds and other animals in these models. Estimates of bycatch (or catch for targeted species) for different species are not independent, and the approach for one species presented here is easily generalized to vectors of species. The estimator for the vector of catch (or bycatch) of a number of species is similar to that used for age or length composition of targeted species discussed by Miller and Skalski (2006). Just as our single-species estimates can be incorporated in a single-species population model, mortality vector estimates and associated variance–covariance matrices for various species can be made and incorporated in a multispecies model.

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List of symbols

- c_v Number of observed fishing trips during the vth vessel-quarter
- C_v Number of fishing trips during the vth vessel-quarter
- g_m Number of hauls sampled in the mth region and (or) period
- g_{mt} Number of hauls sampled in the mth region and (or) period during the tth trip
- g_t Number of hauls sampled during the tth trip
- G_t Number of hauls made during the tth trip
- G_{mt} Number of hauls made in the mth region and (or) period during the tth trip
- h_k Number of hooks sampled in the kth haul
- h_m Number of hooks sampled in the mth region and (or) period
- h_{mt} Number of hooks sampled during the tth trip in the mth region and (or) period
- h_{mq} Number of hooks sampled in the mth region and (or) period during the qth quarter
- H_k Number of hooks deployed in the kth haul
- H_v Number of hooks deployed in the vth vessel-quarter
- H_{mt} Number of hooks deployed during the tth trip in the mth region and (or) period
- n_k Number of mortalities observed from the hooks sampled in the kth haul
- n_m Number of mortalities observed from all hooks sampled in the mth region and (or) period
- N_k Total bycatch in the kth haul
- N_{mt} Total bycatch in the mth region and (or) period in the tth trip
- N_t Total bycatch in the tth trip
- N_v Total bycatch in the vth vessel-quarter
- N_U Total bycatch in the vessel-quarters with 30% observer coverage and only one observed trip
- p_k Probability of capture on a given hook from the kth haul
- p_m Probability of capture on a hook in the mth region and (or) period
- S_t^2 Variance among haul-specific bycatches in the tth trip
- S_v^2 Variance among trip-specific bycatches in the vth vessel-quarter
- V Total number of longline vessel-quarters
- V_M Number of vessel-quarters for vessels in the medium size class
- V_U Number of vessel-quarters with 30% observer coverage and only one observed trip
- ξ_v Inverse of the expected proportion of trips sampled for the vth vessel-quarter
- σ_v^2 Variance parameter for the distribution of the number of observed trips in a given vessel-quarter
- τ_m Dispersion parameter for a model describing the probability of capture on a hook deployed in the mth region and (or) period

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PERSPECTIVE / PERSPECTIVE

Bioenergetics and mercury dynamics in fish: a modelling perspective[1]

Marc Trudel and Joseph B. Rasmussen

Abstract: The concentration of mercury in fish generally increases with age and size. Although a number of hypotheses have been invoked to explain this pattern, our understanding of the processes regulating the accumulation of mercury in fish is currently inadequate. In this study, we used a simple mass balance model to explore how the relationship between mercury concentration and fish age is affected by bioenergetics processes and prey contamination. We show that mercury concentration increases with fish age when older fish consume more contaminated prey or when metabolic costs associated with activity also increase with fish size. Our analyses further indicate that the relative importance of growth rate, activity costs, and consumption rates for mercury concentration can vary widely. We also show that changes in the energy density of fish and their prey with fish size could also affect the relationship between mercury concentration in fish and age. Application of this mass balance model indicates that bioenergetics models underestimate the activity costs of lake trout. A simple approach is presented to estimate activity costs of fish under field conditions.

Résumé : Les teneurs en mercure augmentent généralement en fonction de l'âge et de la taille chez les poisons. Bien qu'un certain nombre d'hypothèses aient été invoquées pour expliquer ces observations, notre compréhension des processus qui régissent l'accumulation du mercure chez les poissons est présentement inadéquate. Dans cette étude, nous utilisons un bilan massif simple pour explorer comment la relation entre les teneurs en mercure et l'âge des poissons est affectée par les processus bioénergétiques et la contamination des proies. Nous démontrons que la teneur en mercure des poisons augmente avec l'âge lorsque les poisons les plus âgés se nourrissent de proies qui sont davantage contaminées ou lorsque les coûts métaboliques associés à l'activité augmentent avec la taille des poissons. De plus, nos analyses indiquent que l'effet relatif de la croissance, des coûts associés à l'activité, et des taux de consommation sur les teneurs en mercure des poissons varient grandement. Nous démontrons également que les changements au niveau de la densité calorique des poissons et de leurs proies peuvent affecté la relation entre les teneurs en mercure et l'âge des poissons. Une application de ce bilan massique indique que les modèles bioénergétiques sous-estiment les coûts associés à l'activité des touladis. Nous présentons une approche simple pour estimer les coûts associés à l'activité des poissons en milieu naturel.

Introduction

Emissions of mercury (Hg) to the atmosphere have increased several-fold since the 19th century (Meili 1995; Schuster et al. 2002). Because its volatility and long residence time in the atmosphere (Dastoor and Larocque 2004), Hg can be dispersed and deposited thousands of kilometres from its original source by atmospheric circulation. As a result, elevated Hg concentrations have not only been observed in fish inhabiting aquatic ecosystems receiving effluents contaminated with Hg, but also in areas that are remote from any direct sources of Hg pollution (Håkanson et al. 1988; Wren et al. 1991; Cabana et al. 1994). Despite recent efforts to reduce industrial emissions of Hg (Schuster et al. 2002; Hylander and Meili 2003), elevated concentrations of Hg will persist for decades in a large number of fish populations

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because of the long half-life of Hg in aquatic ecosystems (Lodenius 1991; Håkanson 1996).

Fish represent the primary source of Hg to human populations (World Health Organization (WHO) 1990). In sufficient quantities, Hg can cause severe neurological damage to humans (e.g., Minamata disease) and retard mental development of children and may even lead to death (Hunter et al. 1940; Bakir et al. 1973). In addition, Hg can be transferred to the foetus during pregnancy and might interfere with the brain development of embryos (WHO 1990). Consequently, the health risk associated with Hg poisoning from the consumption of Hg-contaminated fish and the global Hg contamination of aquatic ecosystems requires an understanding of the factors affecting Hg accumulation in fish.

Mercury concentration in fish is highly variable among individuals, populations, and species (Wren et al. 1991; Simoneau et al. 2005). Within a given population, Hg concentration generally increases with age or size in fish (Somers and Jackson 1993; Tremblay et al. 1998; Sonesten 2003). This is commonly believed to result from the longer exposure time of older fish to Hg (Qian et al. 2001). Clearly, Hg accumulation can take place only when fish are exposed to a source of Hg. However, additional factors besides exposure time are required to explain the lifetime accumulation of Hg in fish, as Hg concentration does not always increase with fish age and size (Scott and Armstrong 1972; Meili 1991).

Theoretical models of contaminant dynamics suggest that Hg concentration increases over time when Hg intake exceeds Hg elimination and growth (Thomann 1981). This may occur if older fish are feeding on more contaminated prey and (or) if food conversion efficiency (i.e., the ratio of growth to consumption rates) decreases with body size (Borgmann and Whittle 1992; Rowan et al. 1998). Alternatively, among-population differences in the rate of Hg accumulation in fish could be simply attributed to growth dilution (Doyon et al. 1998; Stafford and Haines 2001). Yet laboratory studies indicate that contaminant concentration can be higher in faster-growing fish (Spigarelli et al. 1983; Dutton 1997). Clearly, our understanding of the processes regulating the accumulation of Hg in fish is currently incomplete.

The objectives of this study are to provide a quantitative framework to examine the dynamics of Hg accumulation in fish. Using a simple mass balance model, we explore the potential effects of bioenergetics processes associated with feeding, growth, and metabolic rates, as well as prey contamination on Hg dynamics, and assess their effects on the relationship between Hg concentration and fish age. We first derive a Hg mass balance model to show that Hg concentration increases with fish age when older fish consume more contaminated prey or when metabolic costs associated with activity (e.g., foraging, predator avoidance, mating) also increase with fish size. We further show that for a given growth rate, Hg concentration can vary, depending on the combination of food consumption rates and activity costs that produced this growth. We then apply this model to Lake Ontario lake trout (*Salvelinus namaycush*) to illustrate how changes in prey contamination and energy allocation strategies influence Hg concentration in fish. Despite the observation that older fish feed on more contaminated prey, we show that activity costs of Lake Ontario lake trout must increase with size to adequately describe the accumulation of Hg with fish age. We also show that bioenergetics models underestimate the activity costs of lake trout and present a simple approach to estimate activity costs under field conditions.

Mercury mass balance model

Virtually all the Hg in fish is in the form of methylmercury (Bloom 1989, 1992). Hence, in this study, we used Hg and methylmercury interchangeably for fish and their prey.

Fish exposed to Hg-contaminated food and water accumulate about 1000 times more Hg than fish only exposed to Hg-contaminated water (Fig. 1). This suggests that less than 0.1% of the Hg accumulated in fish is the result of a direct uptake of Hg from water (Trudel et al. 2000). Therefore, assuming that Hg uptake from water is negligible in fish, the accumulation of Hg in a fish can be expressed as (Trudel and Rasmussen 2001)

$$(1) \qquad \frac{dB}{dt} = (\alpha \cdot C_q \cdot I \cdot W) - E \cdot B$$

where B is the total quantity of Hg in fish (i.e., burden; μg), α is the assimilation efficiency of Hg from food (dimensionless), C_q is the concentration of Hg in food (μg·g^{-1}), I is the ingestion rate of fish (day^{-1}), W is the mass of fish (g), and E is the elimination rate of Hg from fish (day^{-1}). Published values of α typically range from 65% to 95%, with a modal value around 80% (Fig. 2). As methylmercury is covalently bound to proteins containing sulphur (Harris et al. 2003), α is expected to reflect the assimilation efficiency of proteins, which is also usually around 80% (Brett and Groves 1979). The elimination rate of methylmercury is a function of fish size and water temperature (Trudel and Rasmussen 1997).

For simplicity, we have assumed that the quantity of Hg lost by the release of Hg-contaminated eggs and sperm during spawning was small compared with the quantity of mercury lost by other routes. Because Hg is associated with the protein matrix rather than lipids in fish (Harris et al. 2003), the quantity of Hg exported from body tissues to the gonads is relatively small on a daily basis compared with other Hg losses (Trudel and Rasmussen 2001; Rennie 2003). In addition, Hg mass balance models are not sensitive to this parameter (Trudel and Rasmussen 2001), which suggests that this component can be ignored in the Hg mass balance model. This assumption likely does not hold for lipophilic contaminants such as polychlorinated biphenyls (PCBs) and dichloro-diphenyl-trichloroethane (DDT) (Niimi 1983; de Bruyn et al. 2004). However, the conclusions obtained in this study would not be affected if a parameter for Hg losses due to spawning was included into the mass balance model.

Advisory limits for human consumption are based on Hg concentration in the edible portion of fish (i.e., skinless fillets) rather than on Hg burden in fish. Thus, the Hg mass balance model should predict Hg concentration instead of the total quantity of Hg in fish. Assuming that the concentrations of Hg in muscle tissue and in the whole body are similar in fish (Becker and Bigham 1995; Post et al. 1996), the mass balance model of Hg concentration (C; μg·g^{-1}) can be

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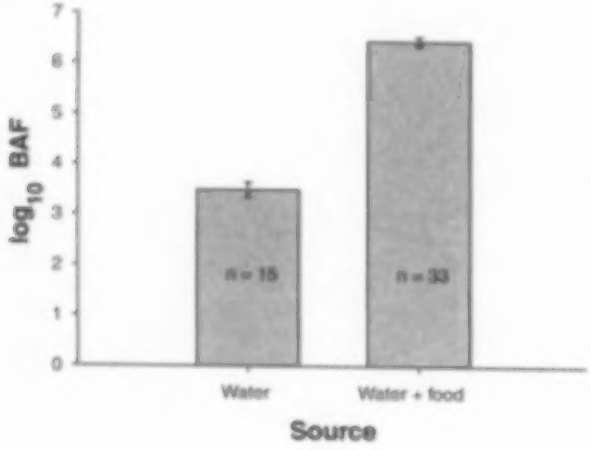
Fig. 1. Bioaccumulation factor (BAF) of methylmercury in fish in relation to the source of contamination. Fish that were only exposed to methylmercury-contaminated water were raised under laboratory conditions, whereas fish that were exposed to both methylmercury-contaminated water and food were collected in the field. Only the dissolved fraction of methylmercury in the water was considered for these calculations. BAFs are likely to be underestimated under natural conditions, as a proportion of the dissolved methylmercury may form ligands with humic substances (Hintelmann et al. 1995) and may not be available to the fish. The error bars represent ± 2 standard errors. BAF was significantly larger for fish exposed to contaminated water and food ($F_{[1,46]}$ = 317.2, $p < 0.0001$; analysis performed on $\log_{10}$-transformed data). BAFs were extracted from Lock (1975), McKim et al. (1976), Pentreath (1976*a*, 1976*b*), Cember et al. (1978), Gill and Bruland (1990), Watras and Bloom (1992), Porcella (1994), Becker and Bigham (1995), Hill et al. (1996), and Bowles et al. (2001).



written by differentiating a quotient as (Trudel and Rasmussen 2001)

$$(2)\qquad \frac{dC}{dt} = \frac{d(B/W)}{dt} = \frac{W \cdot dB - B \cdot dW}{W^2 \cdot dt}$$

$$= \frac{1}{W} \cdot \frac{dB}{dt} - C \cdot \frac{dW}{W \cdot dt}$$

Therefore, the mass balance model of Hg concentration requires a mass balance model for Hg burden, as well as a mass balance model for fish growth. Assuming that fish mass is growing exponentially during the time interval, the mass balance model of fish growth can be written as (Ricker 1979)

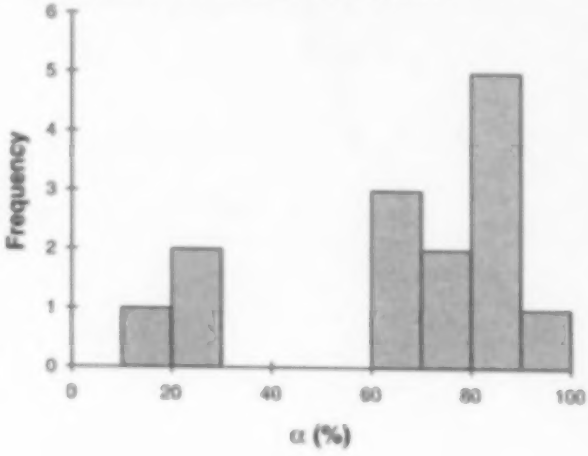
$$(3)\qquad \frac{dW}{W \cdot dt} = G$$

where G is the specific growth rate (day^{-1}). Thus, combining eqs. 1, 2, and 3 gives

$$(4)\qquad \frac{dC}{dt} = (\alpha \cdot C_\delta \cdot I) - (E + G) \cdot C$$

Integrating this equation over time (t, days), we obtain

Fig. 2. Assimilation efficiency of methylmercury (α; %) of fish fed a single dose of methylmercury-contaminated prey fish. Data were extracted from de Freitas et al. (1974), Suzuki and Hatanaka (1975), and Phillips and Gregory (1979).



$$(5)\qquad C_t = C_0 \cdot e^{-(E+G)t} + \frac{\alpha \cdot C_\delta \cdot I}{E+G}[1 - e^{-(E+G)t}]$$

where C_0 is the initial concentration of Hg in fish ($\mu g \cdot g^{-1}$) and C_t is the concentration of Hg in fish ($\mu g \cdot g^{-1}$) at time t (days).

Application of this mass balance model using Hg concentration determined on muscle tissues assumes that Hg concentrations in the whole fish and muscle are similar (Trudel and Rasmussen 2001). The validity of this assumption is uncertain though, as there is evidence both for (Becker and Bigham 1995; Post et al. 1996) and against this assumption (Goldstein et al. 1996; Greenfield et al. 2001). However, there is not sufficient information in these studies to assess the cause of these differences. For instance, the inclusion or exclusion of gonads may affect the estimation of whole-body Hg concentrations, as Hg concentration in female gonads is substantially smaller than in muscle tissues (Hammerschmidt et al. 1999; Trudel et al. 2000). Unfortunately, except for Post et al. (1996), who worked on young-of-the-year yellow perch (*Perca flavescens*), the sex and maturity status of the fish collected in these studies is unknown. It is important to note though that the conclusions obtained in this study are not affected by the validity of this assumption, as eq. 5 can be easily adjusted by multiplying C_0 and C_t by the ratio of whole-body to muscle Hg concentrations when Hg is determined in muscle tissues.

At steady state (i.e., when $dC \cdot dt^{-1} = 0$), this equation simplifies to (Thomann 1981)

$$(6)\qquad C = \frac{\alpha \cdot C_\delta \cdot I}{E + G}$$

The time required to reach this asymptote is determined by $[1 - e^{-(E+G)t}]$; this steady state is expected to be reached faster in fish that are quickly eliminating Hg and growing faster. Although a steady state will be reached only when the parameters of eq. 6 remain constant over time, Hg concen-

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tration will nevertheless track this steady state, provided that the parameters of eq. 6 do not vary too quickly. Thus, to understand why Hg concentration increases with fish age, it is necessary to examine how C_d, I, E, or G vary with fish age or size. As α was not expected to vary systematically with fish size, it was not considered further in this analysis.

Prey contamination

The Hg mass balance model predicts that Hg concentration in fish increases linearly with the concentration of Hg in their food. This prediction is supported both experimentally (Phillips and Buhler 1978) and empirically (MacCrimmon et al. 1983; Allard and Stokes 1989; Cope et al. 1990). Applications of Hg mass balance models also generally indicate that Hg concentration in fish is highly responsive to prey contamination (Harris and Bodaly 1998; Stafford and Haines 2001). Thus, Hg concentration in fish may increase with age when older fish are consuming more contaminated prey, either because they are eating larger and more contaminated prey or because of ontogenic diet and trophic shifts (e.g., plankton to prey fish). This interpretation may not always be sufficient by itself to explain why Hg concentration in fish increases with age, as the ratio of Hg concentration in fish to Hg concentration in their food, also known as the biomagnification factor (BMF), can vary with fish age. For instance, although older Lake Ontario lake trout consume more contaminated prey, the BMF also increases with age in this population (Table 1). Thus, in addition to prey contamination, other factors such as bioenergetics processes may also vary with fish age and influence how much Hg is accumulated in fish through time (Trudel and Rasmussen 2001).

Fish bioenergetics

The effects of bioenergetics processes on the accumulation of Hg in fish can be assessed independently of those associated with prey contamination by dividing both sides of eq. 6 by C_d as (Thomann 1981; Rowan et al. 1998)

$$\text{BMF} = \frac{C}{C_d} = \frac{\alpha \cdot I}{E + G} \quad (7)$$

where BMF is the biomagnification factor (dimensionless) This steady-state equation indicates that Hg concentration may also increase with size if I increases faster with body size than the sum of E and G. Because both I and E tend to scale to body mass with an allometric exponent close to –0.2 (Trudel and Rasmussen 1997; Trudel et al. 2000), Hg concentration will increase with body size if specific growth rate decreases faster with body size than specific feeding rate. In other words, Hg concentration will increase with body size if gross growth efficiency decreases with body size (Borgmann and Whittle 1992; Rowan et al. 1998). A reduction in gross growth efficiency with fish age can occur if metabolic costs are relatively higher in older or larger fish (Pazzia et al. 2002). This equation thus suggests that the concentration of Hg in fish depends not only on the quantity of food consumed by fish, but also on how fish allocate the consumed energy to metabolic costs and growth.

To further understand how Hg dynamics is regulated by bioenergetics processes in fish, it is necessary to examine the process of growth in more detail. Growth occurs when the energy consumed by fish exceeds the energy lost through respiration and waste production such that (Kitchell et al. 1977)

$$G = I - (\text{ACT} \cdot \text{SMR} + \text{SDA} + F + U) \quad (8)$$

where SMR is the standard metabolic rate of fish (day^{-1}), SDA is the specific dynamic action (day^{-1}), F and U are the fecal and nitrogen wastes (day^{-1}), respectively, and ACT represents activity costs and is expressed here as a multiple of SMR (further referred to as the activity multiplier). For simplicity, we have assumed that the energy density of fish and their prey were the same, though this assumption will be subsequently relaxed (see Energy density and Hg dynamics section). SMR is a function of fish mass and water temperature, whereas SDA, F, and U vary as a function of food consumption rates (Hanson et al. 1997; Clarke and Johnston 1999). The activity multiplier is commonly assumed to be constant across populations and size and is often assumed to be equal to 1 in several species, including northern pike (*Esox lucius*) and largemouth bass (*Micropterus salmoides*) (Hanson et al. 1997). This value implies that the amount of energy allocated to external activity is negligible in these species. Alternatively, SMR is often based on routine metabolic costs that include some levels of activity (Trudel and Boisclair 1994; Aubin-Horth et al. 1999). In this case, an activity multiplier of 1 indicates that fish are not expending energy to activity beyond what is used in routine metabolic rates.

As the sum of SDA, F, and U typically represents a constant proportion of food consumption rates over a wide range of temperatures and feeding rates (Kitchell et al. 1977; Boisclair and Leggett 1989; but see Bajer et al. 2004), eq. 8 can be simplified to

$$G = \tau \cdot I - \text{ACT} \cdot \text{SMR} \quad (9)$$

where τ represents the proportion of food that can be metabolized (i.e., $\tau = 1 - [\text{SDA} + F + U] \cdot I^{-1}$).

Substituting eq. 9 into eq. 7, we obtain

$$\text{BMF} = \frac{\alpha \cdot I}{E + \tau \cdot I - \text{ACT} \cdot \text{SMR}} \quad (10)$$

As with I and E, SMR tends to scale to body mass with an allometric exponent close to –0.2 (Hanson et al. 1997; Clarke

Table 1. Mercury concentration in Lake Ontario lake trout (*Salvelinus namaycush*) ([Hg]; μg·g wet weight^{-1}) and their food (C_d; μg·g wet weight^{-1}) in relation to mass (W; g).

Age	Maturity	W	[Hg]	C_d	BMF
1	Juvenile	68	0.03	0.023	1.3
2	Juvenile	322	0.06	0.027	2.2
3	Juvenile	939	0.10	0.031	3.3
4	Juvenile	1690	0.12	0.034	3.6
5	Adult	2414	0.15	0.037	4.1
6	Adult	3090	0.18	0.040	4.6
7	Adult	3723	0.20	0.043	4.7
8	Adult	4320	0.21	0.045	4.7

Note: Data are from Borgmann and Whittle 1992. BMF, biomagnification factor.

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and Johnston 1999). Therefore, Hg concentration will increase with body size if ACT is scaled to body size with an allometric exponent greater than zero (i.e., ACT $\propto W^b$, with $b > 0$). Thus, Hg concentration will increase with body size if activity costs also increase with size (Trudel and Rasmussen 2001). The interpretation that activity costs increase with body size in fish is supported by field studies (Boisclair and Rasmussen 1996; Rowan and Rasmussen 1996; Sherwood et al. 2002).

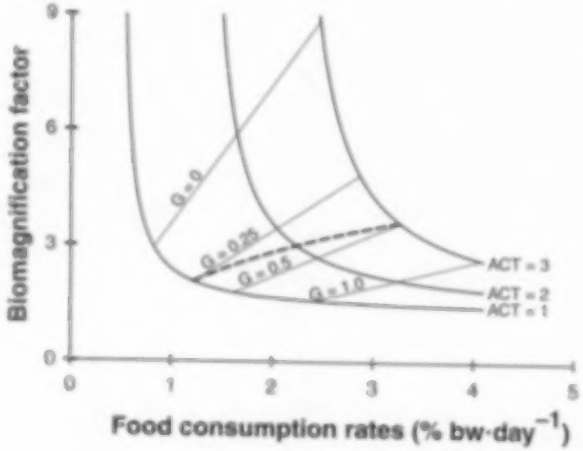
From eq. 10, it is clear that for a given level of feeding rate and prey contamination, a decrease in activity costs (or multiplier) would lead to a reduction in Hg concentration in fish. However, it is more difficult to assess the effects of increasing food consumption rates on Hg concentration in fish without any visual aid, as this parameter appears on both the numerator and denominator of eq. 10. We plotted the BMF of a hypothetical 100 g lake trout at 10 °C in relation to food consumption rates and activity costs, as well as growth rates (Fig. 3). This graph indicates that for a given activity multiplier and prey contamination, increasing food consumption rates would also reduce Hg concentration in fish, provided that growth is positive. The largest change in BMF is produced when growth is close to zero and activity costs are high. When food consumption rates are high, the model becomes less sensitive to changes in activity costs. A similar pattern was generated using fish of different sizes, though the curves observed in Fig. 3 would be shifted to the left or to the right in larger and smaller fish, respectively.

τ may also increase with size for fish undergoing an ontogenic diet shift from invertebrates to prey fish and thus affect the relationship between Hg concentration and fish age. However, eq. 10 predicts that an increase in τ would result in a reduction of BMF. Therefore, a diet shift to more digestible prey cannot account for the increase in Hg concentration with fish age.

Growth dilution

It has frequently been argued that, all else being equal (i.e., the "ceteris paribus" condition), Hg concentration should be lower in fast-growing fish than in slow-growing fish because the fast-growing fish can dilute the ingested Hg into a larger mass (Doyon et al. 1998; Essington and Houser 2003; Simoneau et al. 2005). The growth dilution hypothesis has also been invoked to explain patterns in accumulation of other persistent contaminants as well, such as PCBs (Stow and Carpenter 1994). However, Dutton (1997) also showed that under laboratory conditions, Hg concentration was higher in faster-growing fish, indicating that growth is not sufficient by itself to explain patterns of contaminant accumulation in fish. The simulations performed in this study also indicate that Hg concentration in fish can vary for a given growth rate depending on the combination of food consumption rates and activity costs that produced this growth (Fig. 3). For instance, at a growth rate equal to 0.5% body weight·day^{-1}, Hg concentration in lake trout would be twofold higher if ACT was equal to 3 compared with if it was equal to 1. In addition, this graph indicates that Hg concentration could even be larger in fast-growing fish, depending on how much food fish consumed and how much energy fish allocated to activity. An appropriate interpretation of the growth dilution hypothesis is that for a given food consumption rate, fish with the faster growth rate will reach a lower Hg concentration than the slow-growing fish (Borgmann and Whittle 1992; Rowan et al. 1998). Thus, Hg concentration is expected to decrease with increasing growth efficiency. In other words, fish with the lowest activity costs are expected to reach lower levels of Hg contamination.

Fig. 3. Biomagnification factor for a 100 g lake trout (*Salvelinus namaycush*) at 10 °C in relation to food consumption rates (% body weight (bw)·day^{-1}), activity multiplier (ACT; dimensionless), and growth rates (G; % bw·day^{-1}). The broken line was obtained by assuming that activity costs were positively correlated to consumption rates.



The Hg mass balance model can be used to explore the concept of growth dilution and examine how Hg concentration is affected by growth rates and growth efficiency. We plotted the BMF of a hypothetical 100 g lake trout at 10 °C in relation to growth rates and growth efficiency, as well as activity costs (Fig. 4). This graph indicates that for a given activity multiplier and prey contamination, a reduction of growth rates or growth efficiency with size through a reduction in consumption rates would result in an increase in Hg concentration in fish. Similarly, for a given consumption rate and prey contamination, a reduction in growth rates or growth efficiency with size through an increase in activity costs would result in an increase in Hg concentration in fish (Figs. 3, 4). The relationships between BMF and growth rates and between BMF and growth efficiency were steep at low values of growth rates and growth efficiency, but approached an asymptote at high values of growth rates and growth efficiency. Thus, all else being equal, at steady state, Hg concentration should be lower in faster-growing fish or in fish with higher growth efficiency. However, our analyses also indicate that the effects of growth and growth efficiency on Hg concentration may be obscured by how much food fish consume and how much of their energy budget they allocate to activity. For instance, different BMFs can be produced at a given growth rate or growth efficiency depending on the combination of consumption rates and activity costs. Hence, despite the fact that the mass balance model presented in this study included a term for growth dilution, our analyses indicate that the Hg accumulation in fish is not a

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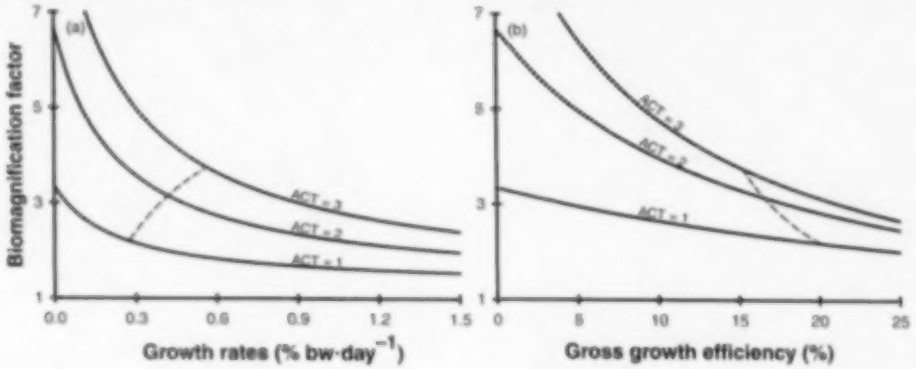
Fig. 4. Biomagnification factor (BMF) for a 100 g lake trout (*Salvelinus namaycush*) at 10 °C in relation to growth rates, growth efficiency, and activity multiplier (ACT; dimensionless). The broken line was obtained by assuming that activity costs were positively correlated to consumption rates. bw, body weight.



simple function of growth or growth efficiency. This may explain why the evidence supporting the growth dilution hypothesis has been equivocal.

Energy density and Hg dynamics

Energy density generally increases with size in fish (Hanson et al. 1997; Trudel et al. 2005). As a consequence, a lower mass is gained in older and larger fish for a given prey energy density (Stewart et al. 1983). Moreover, the ratio of energy density of fish to the energy density of their prey may increase with age, which violates the assumption of the Hg mass balance model presented in this study. Hence, the energy density of the prey and predator should be incorporated into the Hg mass balance model. This can be achieved by converting eq. 9 to kJ·day^{-1} as

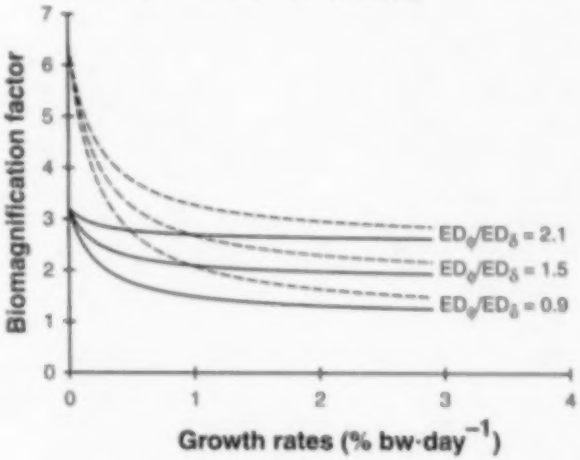
$$(11)\quad G \cdot \mathrm{ED}_\phi = \tau \cdot I \cdot \mathrm{ED}_\delta - \mathrm{ACT} \cdot R_s \cdot Q_{ox}$$

where ED_ϕ and ED_δ are the energy density of fish and their food (both in kJ·g^{-1}), respectively, R_s is the standard metabolic rate (g O_2·g^{-1}·day^{-1}), and Q_{ox} is an oxycalorific equivalent equal to 13.56 kJ·g O_2^{-1} (Elliott and Davison 1975). Substituting eq. 11 into eq. 7 and simplifying:

$$(12)\quad \mathrm{BMF} = \frac{\alpha \cdot I}{E + \tau \cdot I \cdot \dfrac{\mathrm{ED}_\delta}{\mathrm{ED}_\phi} - \mathrm{ACT} \cdot R_s \cdot \dfrac{Q_{ox}}{\mathrm{ED}_\phi}}$$

This steady-state equation predicts that BMF increases with an increase in the ED_ϕ–ED_δ ratio (Fig. 5). However, the sensitivity of BMF to energy density varies in relation to fish growth. At low growth, BMF is relatively insensitive to changes in ED_ϕ, whereas BMF is proportional to the ED_ϕ–ED_δ ratio in fast-growing fish (Fig. 5). This occurred because the BMF depends only on food consumption rates and elimination rates when growth is equal to zero. In this special case, BMF is equal to $\alpha \cdot I \cdot E^{-1}$ and is thus independent of both ED_ϕ and ED_δ. BMF is also expected to decrease with increasing growth and to be higher in more active fish (Fig. 5). Thus, Hg concentration may increase with fish age simply because of an increase in fish energy density relative to that of its prey, combined with a reduction in specific growth rates, either through a reduction in food consumption rates or an increase in activity costs with age (Figs. 3–5).

Fig. 5. Biomagnification factor (BMF) for a 100 g lake trout (*Salvelinus namaycush*) at 10 °C in relation to growth rates obtained using three different ratios of fish energy density (ED_ϕ; kJ·g^{-1}) to prey energy density (ED_δ; kJ·g^{-1}). ACT = 1.0, solid line; ACT = 2.0, broken line; bw, body weight.



Model application

To examine the effects of prey contamination and bioenergetics processes on Hg concentration in fish, we modeled the accumulation of Hg in Lake Ontario lake trout by combining eq. 5 with the bioenergetics model of lake trout proposed by Stewart et al. (1983). We performed a series of simulations using three scenarios for activity costs. In the first scenario, we tested the assumption that activity costs are negligible in lake trout by setting the activity multiplier (ACT) equal to 1. This simulation also tested the hypothesis that changes in prey contamination were sufficient to explain the pattern of Hg concentration with lake trout age. In the second scenario, we used the ACT function assumed by Stewart et al. (1983) for lake trout. This simulation tested

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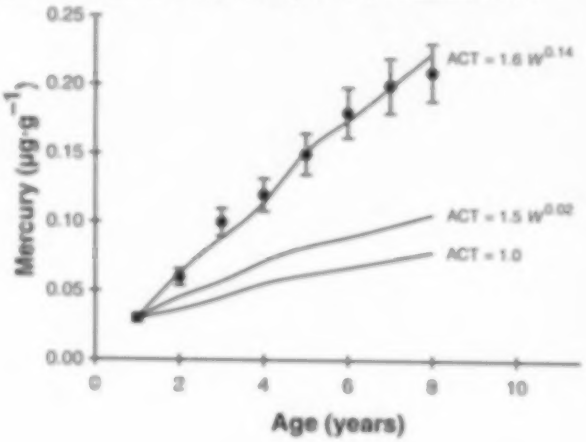
the validity of the activity costs assumed by Stewart et al. (1983). Finally, in the last scenario, we modeled ACT as a power function of size and adjusted the coefficients of this function until we obtained a reasonable fit between observed and predicted Hg concentration in Lake Ontario lake trout (Rennie et al. 2005*a*). For all the simulations, C_0 and W_0 were set as the concentration and size, respectively, of age-1+ lake trout (Table 1). The temperature of Lake Ontario was modeled using a Gaussian function (Trudel et al. 2000) and the data collected by Kwiatkowski (1982). The energy density of the prey fish consumed by lake trout was taken from Rottiers and Tucker (1982), whereas the energy density of lake trout was modelled as a function of lake trout size following Stewart et al. (1983). We further assumed that (*i*) the maximum preferred temperature of lake trout was 10 °C (Pazzia et al. 2002), (*ii*) the energy density of the gonads was 20% higher than the energy density of somatic tissue (Diana 1983), and (*iii*) α was equal to 80% (Fig. 2; Trudel and Rasmussen 2001).

The simulations showed that Hg concentration reached a quasi steady state in Lake Ontario lake trout when activity costs were assumed to be negligible (Fig. 6). Furthermore, the mass balance model significantly underestimated the accumulation of Hg in Lake Ontario lake trout when the activity costs assumed by Stewart et al. (1983) were used in the lake trout bioenergetics model. Thus, despite the fact that larger lake trout consumed more contaminated prey in Lake Ontario, it was not sufficient to explain why Hg concentration increased with fish age in this population. Instead, the simulations performed in this study strongly suggest that the dynamics of Hg concentration in fish will be highly dependent on energy acquisition and allocation strategies. In particular, these results suggest that Hg concentration increases with fish age and size because of a higher allocation of energy to activity costs as the fish get larger. These simulations further suggest that the allometry of activity costs for this population is higher than that predicted by Stewart et al. (1983). Although the bioenergetics model of Stewart et al. (1983) may provide reasonable estimates of activity costs for some lake trout populations (e.g., Madenjian et al. 2000), it is not currently possible to determine under which circumstances these values will be appropriate (Boisclair and Leggett 1989, 1991; Trudel and Rasmussen 2001). Therefore, unless site-specific estimates of activity costs are available from direct measurements or empirical models developed using field-derived activity costs, predictions obtained with a contaminant mass balance model implemented with a laboratory-derived bioenergetic model should be interpreted cautiously (Trudel and Rasmussen 2001).

Modelling activity costs

Activity costs are particularly difficult to estimate in the field (Trudel and Boisclair 1996; Cooke et al. 2004). Hence, empirical models of activity costs may provide a low cost alternative to direct field measurements (Boisclair and Rasmussen 1996; Sherwood et al. 2002) and may improve Hg mass balance model predictions (Trudel and Rasmussen 2001). As foraging costs are likely to represent the major component of activity in fish (Kerr 1982), it may be possible

Fig. 6. Mercury (Hg) accumulation in Lake Ontario lake trout (*Salvelinus namaycush*) (data from Borgmann and Whittle 1992). The solid lines represent values predicted from mass balance model and a bioenergetics model that was implemented with different functions describing activity multipliers (ACT; dimensionless). The error bars represent ± 2 standard errors.



to derive a relationship between activity costs and food consumption rates as

$$\text{ACT} = \beta_0 + \beta_1 \frac{I \cdot \text{ED}_\delta}{R_s \cdot Q_{\text{os}}} \quad (13)$$

where β_0 and β_1 are empirically derived constants. Substituting eq. 13 into eq. 11 and simplifying:

$$G \cdot \text{ED}_\phi = (\tau - \beta_1) \cdot I \cdot \text{ED}_\delta - \beta_0 \cdot R_s \cdot Q_{\text{os}} \quad (14)$$

Growth rates and energy densities can be estimated directly from field observations. In addition, R_s and τ are readily available for a number of fish species (Hanson et al. 1997; but see Trudel et al. 2004). Hence, bioenergetics models can be simplified to one equation with one unknown with an empirical model of activity costs and solved for consumption rates as

$$I = \frac{G \cdot \text{ED}_\phi + \beta_0 \cdot R_s \cdot Q_{\text{os}}}{(\tau - \beta_1) \cdot \text{ED}_\delta} \quad (15)$$

Unfortunately, independent estimates of activity costs and consumption rates are currently not available to determine the values of β_0 and β_1 and to evaluate the performance of eq. 15. For instance, Pazzia et al. (2002) estimated the activity costs of lake trout as the difference between food consumption rates and the sum of growth rates, standard metabolic rates, waste production, and specific dynamic action. The data provided by Pazzia et al. (2002) may nevertheless be used to illustrate how eqs. 13–15 can be implemented as independent estimates of activity costs and consumption rates become available.

The activity costs derived by Pazzia et al. (2002) were positively correlated to consumption rates in lake trout, though this relationship differed significantly between piscivorous and nonpiscivorous lake trout ($F_{[1,55]} = 19.5$, $p < 0.0001$; Fig. 7). These relationships were then incorporated

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Fig. 7. Relationship between the activity multiplier (ACT; dimensionless) and the ratio of consumption rates (I; kJ·day^{-1}) to standard metabolic rates (SMR; kJ·day^{-1}) in piscivorous (open circles) and nonpiscivorous (solid squares) lake trout (*Salvelinus namaycush*) (data from Pazzia et al. 2002). Consumption rates were standardized to standard metabolic rates to make them comparable with activity multipliers.

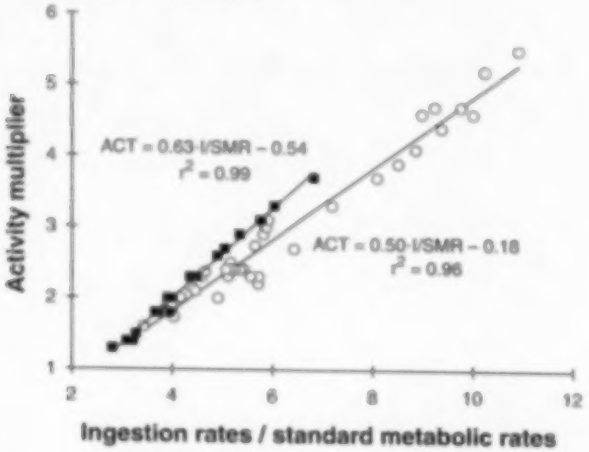
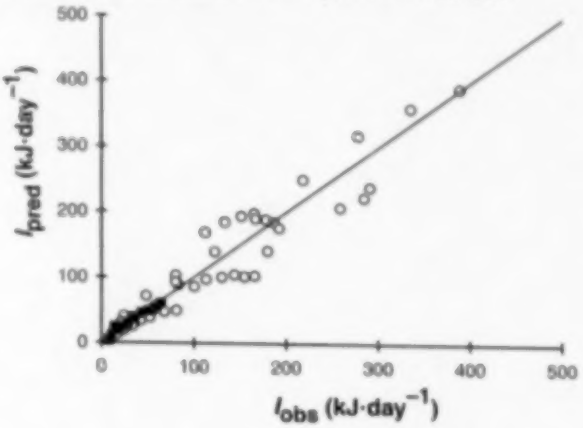


Fig. 8. Relationship between consumption rates of lake trout (*Salvelinus namaycush*) predicted with a bioenergetics model that incorporates an empirical model for activity costs (I_{pred}; kJ·day^{-1}) with consumption rates derived by Pazzia et al. (2002) (I_{obs}; kJ·day^{-1}) for piscivorous (open circles) and nonpiscivorous (solid squares) lake trout. The solid line represents the 1:1 line.



directly into the lake trout bioenergetics model, which simplified to

$$G \cdot ED_\phi = 0.12 \cdot I \cdot ED_\delta + 0.18 \cdot R_s \cdot Q_{ox} \quad (16)$$

for piscivorous lake trout and to

$$G \cdot ED_\phi = -0.05 \cdot I \cdot ED_\delta + 0.54 \cdot R_s \cdot Q_{ox} \quad (17)$$

for nonpiscivorous lake trout. These models provided reasonable estimates of consumption rates (Fig. 8). More than 70% of the predicted values were within 25% of the observed values (range = 0.3%–69.1%). However, it is important to note that this does not constitute a validation of these models, as the same data were used to develop and test the models. This analysis nevertheless verified the internal consistency of these models. Further testing of these models with independent estimates of consumption and growth rates is required to assess their validity.

Empirical models of activity costs may also be derived using environmental conditions and glycolitic enzymes. For instance, Boisclair and Rasmussen (1996) derived a relationship between the activity costs of yellow perch with water clarity and amphipod biomass, and Sherwood et al. (2002) showed that activity costs in this species were strongly correlated to the biochemical activity of lactate dehydrogenase. Unlike the approach presented in this study, these empirical models of activity costs require the estimation of additional variables in the field or in the laboratory. Thus eqs. 14 and 15 represent a step further at simplifying bioenergetics models. These equations may also be applicable to other species for which activity costs and consumption rates have been derived.

The relationships derived in this study between activity costs and consumption rates can be used to constrain the range of biologically plausible combinations for these parameters and, hence, to predict how Hg concentration in lake trout is likely to vary in relation to age, size, growth rates, and growth efficiency. This can be achieved by substituting eq. 14 into eq. 7 as

$$BMF = \frac{\alpha \cdot I}{E + (\tau - \beta_1) \cdot I \cdot \frac{ED_\delta}{ED_\phi} - \beta_0 \cdot R_s \cdot \frac{Q_{ox}}{ED_\phi}} \quad (18)$$

or alternatively as (when growth rates are available instead of consumption rates)

$$BMF = \frac{\alpha(G \cdot ED_\phi + \beta_0 \cdot R_s \cdot Q_{ox})}{(\tau - \beta_1)(E + G)ED_\delta} \quad (19)$$

Equation 16 was used to generate growth rates and gross growth efficiencies of a 100 g piscivorous lake trout at 10 °C over a range of food consumption rates (1.2%–3.3% body weight·day^{-1}). For these analyses, we set ED_δ equal to ED_ϕ (5.0 kJ·g^{-1}). We then used these values to estimate lake trout BMF using eq. 18. These analyses predict that Hg concentration in lake trout would be positively correlated to both consumption rates and growth rates, but negatively correlated to gross growth efficiency (Figs. 3–4). These results occurred despite that the Hg mass balance model used in this study assumes that changes in Hg concentration over time are negatively related to growth. These results are also consistent with those obtained by Dutton (1997) in a series of laboratory experiments performed on rainbow trout (*Oncorhynchus mykiss*) in which he showed that Hg concentration increased at a higher rate in faster-growing fish. In addition to Hg, theoretical models predict that the accumulation of persistent organic contaminants is inversely correlated to growth efficiency throughout the animal kingdom (de Bruyn and Gobas 2006). It is important to note though that the analyses presented above invoked the "ceteris paribus" condition. Hence, these relationships may be masked across popu-

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lations by several factors, including differences in body size, water temperature, prey quality, and fish energy density.

Inverse modelling

Mass balance models can, in principle, be solved for any variables provided that the number of unknown variables does not exceed the number of equations. For instance, the Hg mass balance model presented in this study has been used to estimate food consumption rates of fish using Hg concentration in fish and in their food, fish age and size, and water temperature (Trudel et al. 2001; Rennie et al. 2005*a*). These estimates were obtained assuming that α of Hg was equal to 80% and using the Hg elimination model of Trudel and Rasmussen (1997). With the simplified bioenergetics model presented above, it may also be possible to solve the Hg mass balance model for other variables, such as prey contamination (e.g., Post et al. 1996), or even to derive field estimates of α. In particular, Jackson and Schindler (1996) suggested that mass balance models of contaminant dynamics could be used to estimate the net trophic transfer of contaminants from food (γ; dimensionless) as

$$\gamma = \mathrm{BMF}\left(\frac{G}{I}\right) \quad (20)$$

This equation is obtained by setting E equal to zero in eq. 7. Madenjian et al. (1998*a*, 1998*b*) further suggested that γ was equal to α for slowly eliminated contaminants such as PCBs. This equation assumes (*i*) that the concentration of contaminant in fish is in steady state (i.e., contaminant concentration remains constant over time or $dC \cdot dt^{-1} = 0$), (*ii*) that the elimination rate of the contaminant is negligible with respect to growth, (*iii*) that the energy density of the predator and prey are the same, and (*iv*) that the uptake of the contaminant from water is negligible. However, this equation may not be adequate for estimating α of Hg as three of these assumptions are violated. First, Hg concentration generally increases with fish age, indicating that it is not in steady state. Second, there is no indication that the elimination rate of Hg is negligible with respect to growth in fish. For instance, the elimination rate of Hg is expected to be approximately 1.5-fold higher than lake trout growth rate for the six populations studied by Pazzia et al. (2002). Thus, even if Hg concentration was in steady state, it is unclear what γ would represent for Hg, as γ would still not be equal to α, but instead

$$\gamma = \alpha\left(\frac{G}{E+G}\right) \quad (21)$$

This equation indicates that unless elimination is negligible, γ will always be lower than α. Thirdly, the energy density of fish generally increases with age and is usually higher than that of their prey (Hanson et al. 1997). This indicates that eq. 20 should not be used to estimate α of Hg. Instead, α may be estimated by iteration by solving eq. 5 using a bioenergetics model that accounts for differences in energy density between fish and their food, such as eq. 14. The validity of the assumptions of eq. 20 should be evaluated for other persistent contaminants as well before it is broadly applied in the field, as this model also assumes that the elimination of persistent contaminants during spawning through the release of contaminated gonads is negligible. This appears to be the case for Hg but may not be valid for persistent organic contaminants such as PCBs (Niimi 1983; de Bruyn et al. 2004).

Summary and conclusions

The Hg mass balance model presented in this study provides a flexible framework for understanding the factors affecting the concentration of Hg in fish. This model predicts that Hg concentration increases with fish age when older fish are feeding on more contaminated prey. As Hg concentration in fish is highly responsive to prey contamination (Harris and Bodaly 1998; Stafford and Haines 2001), further effort is required to understand the factors affecting the concentration of methylmercury in aquatic invertebrates and prey fish. Empirical models of methylmercury concentration in zooplankton and invertebrates may be an important step to assess the relative roles of environmental factors on Hg concentration in lower trophic levels (Westcott and Kalff 1996; Rennie et al. 2005*b*). However, our analyses also indicate that prey contamination may not always be sufficient to explain the within- and among-population variability in Hg concentration in fish. The simulations and graphical analyses performed in this study indicate that Hg concentration also increases with fish age when metabolic costs associated with activity (e.g., foraging, predator avoidance, mating) increase with fish size or when consumption rates decrease with body size because of a decrease in growth efficiency. Furthermore, our analyses indicate that the underlying relationship between Hg concentration and fish growth may be masked by changes in activity costs, even when changes in prey contamination are accounted for. In particular, Hg concentration may be either positively or negatively correlated to changes in growth and growth efficiency, depending on how fish allocate energy between activity costs and growth rates. These results may explain why it has been difficult to find consistent empirical support to the growth dilution hypothesis from field observations. We also showed that changes in the energy density of fish and their prey could also affect the relationship between Hg concentration in fish and age.

Our analyses also indicate that Hg mass balance models may not always accurately predict Hg concentration in fish when they are combined with bioenergetics models because of the inability of bioenergetics models to adequately describe activity costs of fish in natural conditions (Trudel and Rasmussen 2001). This indicates the need to obtain accurate estimates of activity costs of fish under natural conditions. To improve bioenergetics model predictions, we derived an empirical relationship between activity costs and consumption rates. This relationship was based on the assumption that foraging costs represented the major fraction of fish activity (Kerr 1982). However, it is important to note that the activity costs used in this study for piscivorous and nonpiscivorous lake trout were estimated as the difference between consumption rates and the sum of growth and energy expenditures (Boisclair and Leggett 1989). Hence, the activity costs and consumption rates were not independent and were expected to be correlated. Thus, the correlation observed in this study between activity costs and consumption rates may be spurious and should be tested using independ-

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ently derived estimates (Boisclair 1992). Nevertheless, this approach could reduce the number of unknowns in bioenergetics models to a single variable, as size, age, and temperature, as well as the energy of fish and their prey, can be readily determined from field observations.

In contrast to this study, Bajer et al. (2004) suggested that biases in bioenergetics model predictions were primarily attributed to errors associated with the estimation of egestion, excretion, or specific dynamic action. They argued that inaccuracies in activity costs were unlikely to be important in laboratory experiments, as fish activity is limited and constrained by the volume of the tank. However, fish may be able to adjust their activity in relation to the amount of food available, even under laboratory conditions (Kerr 1982). Furthermore, laboratory conditions may not adequately reflect conditions in the wild (Trudel and Welch 2005). In particular, fish are exposed to predators and competitors and may repeatedly be using burst swimming, which has been hypothesized to account for a large amount of activity budgets in fish (Sherwood et al. 2002). Hence, fish in the field may allocate a higher fraction of their energy budget to activity than fish in the laboratory (Boisclair and Leggett 1989). Thus, biases in bioenergetics model prediction may be attributed in part or totally to errors associated with the estimation of activity costs in the field and in the laboratory. Further work is required to determine the source of biases in bioenergetics model predictions and to improve their reliability.

In this study, we focused our analyses primarily on the biomagnification factor rather than Hg concentration in fish, as this approach allowed us to separate the effects of bioenergetics processes to those associated with prey contamination. Biomagnification occurs when the concentration of a chemical increases at each successive trophic level and occurs in chemicals that are efficiently assimilated and slowly excreted (Thomann 1981). Our analyses showed that the biomagnification factor could vary considerably depending on consumption rates and activity costs. Field studies support the hypothesis that the biomagnification factor is not a constant, but rather varies significantly within and among populations (MacCrimmon et al. 1983; Borgmann and Whittle 1992; Watras et al. 1998). Hence, the biomagnification factor may serve as a diagnostic tool to indicate variability in bioenergetics processes (Rowan et al. 1998).

Mathematical models are important for establishing general principles, for generating and testing hypotheses, and for directing future investigations. However, by definition, models are a simplification of reality and will not fully describe natural processes. Hence, the utility of models will depend largely on the accuracy of their predictions. The Hg mass balance model presented in this study has been successfully tested with independent estimates of growth and consumption rates (Trudel et al. 2000; Trudel and Rasmussen 2001), indicating that this mass balance model provides an adequate description of the processes regulating the accumulation of Hg in fish. Nevertheless, further work is required to parameterize this model. Notably, we have assumed that the assimilation efficiency of Hg from food was 80% and did not vary among species and prey (Trudel and Rasmussen 2001). Yet, published estimates of assimilation efficiency range from about 20% to 95%. The empirical model used to estimate the elimination rate of Hg from fish only accounted for 77% of the variation in Hg elimination rate and was assumed to be valid for every species of fish (Trudel and Rasmussen 1997). Furthermore, Hg elimination rates were assumed to be independent of metabolic rates and of Hg concentration in fish. Yet, the allometric exponent and temperature coefficient of Hg elimination are similar to those derived for standard metabolic rates in fish (Clarke and Johnston 1999). Clearly, the validity of these assumptions needs to be tested further.

The tragic episode of Hg poisoning in Minimata has stimulated considerable research into understanding Hg accumulation in fish. This research has considered a wide range of biological and environmental variables, including fish size, trophic position, growth, pH, and water colour (e.g., Wren et al. 1991; Cabana et al. 1994; Greenfield et al. 2001; and many others). Our study indicates a central role of fish bioenergetics in Hg accumulation. A balanced model of energy intake and expenditure, combined with information on prey contamination, allows precise predictions of Hg levels in fish (Trudel and Rasmussen 2001). Unfortunately, the mechanisms regulating bioenergetics processes in wild fish remain poorly understood. In particular, further effort is needed to understand how food consumption rates and activity costs vary with environmental conditions and how these in turn affect the concentration of Hg in fish.

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